

# Current Knowledge of *Trichosporon* spp. and Trichosporonosis

Arnaldo L. Colombo,<sup>1\*</sup> Ana Carolina B. Padovan,<sup>1</sup> and Guilherme M. Chaves<sup>2</sup>

*Laboratório Especial de Micologia, Disciplina de Infectologia, Universidade Federal de São Paulo, São Paulo, Brazil,<sup>1</sup> and  
Laboratório de Micologia Médica e Molecular, Departamento de Análises Clínicas e Toxicológicas,  
Universidade Federal do Rio Grande do Norte, Natal, Brazil<sup>2</sup>*

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## INTRODUCTION

*Trichosporon* spp. are basidiomycetous yeast-like anamorphic organisms (Basidiomycota, Hymenomycetes, Tremelloidaceae, Trichosporonales) that are widely distributed in nature and found predominantly in tropical and temperate areas. These organisms can be found in substrates such as soil, decomposing wood, air, rivers, lakes, seawater, cheese, scarab beetles, bird droppings, bats, pigeons, and cattle. In humans, these fungal species occasionally are part of the gastrointestinal and oral cavity microbiota and can transiently colonize the respiratory tract and skin (22, 60, 68, 76, 101, 108, 170, 186, 188, 189). Recently, Silvestre et al. found that 11% of their 1,004 healthy male volunteers were colonized by *Trichosporon* species on their normal perigenital skin (scrotal, perianal, and inguinal sites of the body) (165).

The genus *Trichosporon* is phenotypically characterized by the ability to form blastoconidia, true mycelia, and, most importantly, arthroconidia, asexual propagules that disarticulate from true hyphae. The presence of multilamellar cell walls and dolipores with or without parenthesomes is an important characteristic of *Trichosporon* spp. (66, 69). Nevertheless, some species possess other morphological structures that can also be used to differentiate them, including appresoria, macroconidia, or other morphological structures and processes. *Trichosporon* spp. are able to utilize different carbohydrates and carbon sources and to degrade urea, but members of this genus are nonfermentative. Cell cultures grow on Sabouraud dextrose agar as yeast colonies with colors ranging from white to cream, typically displaying aspects such as cerebriform and radial surfaces (39). Colonies may become dry and membranous with age (101).

The genus *Trichosporon* has a long and controversial history. It was first designated in 1865 by Beigel, who observed this microorganism causing a benign hair infection. The word *Trichosporon* is derived from the Greek and represents a combination of *Trichos* (hair) and *sporon* (spores). This nomencla-

\* Corresponding author. Mailing address: Laboratório Especial de Micologia, Disciplina de Infectologia, Universidade Federal de São Paulo, São Paulo-SP. Rua Botucatu, 740, CEP 04023-062 São Paulo, Brazil. Phone and fax: (55) (11) 5083-0806. E-mail: colomboal@terra.com.br.

ture is related to the presence of irregular nodules along the body and head hair, consisting of a fungal infection, which is further characterized as white piedra (meaning "stone" in Spanish). Indeed, the etiological agent of this white piedra was formerly misclassified as the alga *Pleurococcus beigelii* (66, 69). In 1890, Behrend described the agent causing white piedra found on a man's beard in detail and named it *Trichosporon ovoides* (16). Since then, other *Trichosporon* species have been reported. In 1902, Vuillemin designated all *Trichosporon* species *Trichosporon beigelii*, an arthrospore-containing yeast (66, 69).

In 1909, Beurmann et al. cultured fungal elements collected from a patient with a pruritic cutaneous lesion and designated the isolated fungus *Oidium cutaneum*, which was subsequently renamed *Trichosporon cutaneum* by Ota in 1926. Nevertheless, in 1942 Diddens and Lodder considered *T. beigelii* and *T. cutaneum* to be the same species. This led to the use of two names for this fungus with clinical relevance: *Trichosporon beigelii*, adopted by physicians, and *Trichosporon cutaneum*, preferred by environmental mycologists (66, 69).

Although most *Trichosporon* spp. routinely isolated in laboratories are related to episodes of colonization or superficial infections, this fungus has been recognized as an emergent opportunistic agent causing invasive infections in tertiary care hospitals worldwide (25).

The genus *Blastoschizomyces*, which contains a unique species named *B. capitatus*, was also considered to belong to the *Trichosporon* genus in the past. It has been recognized as a rare but emerging cause of disseminated infection specifically in leukemic patients (32). *B. capitatus* was thereafter reassigned to the genus *Geotrichum* (*Geotrichum capitatus*), but since 1985 the species has been recognized as more closely related to the Ascomycetes despite the fact it produces blastoconidia and arthroconidia just like *Trichosporon* (157). Indeed, *B. capitatus* has a well-described teleomorph named *Dipodascus capitatus* which produces asci and ascospores (38). In addition, it produces annelloconidia, a distinct cell wall composition, and septal pores, peculiar characteristics that make it different from the genera *Trichosporon* and *Geotrichum* (157).

#### CURRENT TAXONOMY OF *TRICHOSPORON* SPP.

Traditional taxonomy based on phenotypic characterization of fungal organisms generates inconsistent data when applied to the genus *Trichosporon*, as it aggregates within the same taxon isolates with genetically heterogeneous behavior. In 1982, some strains belonging to the taxon *T. beigelii* were found to possess a Q-10 ubiquinone system, while others contained a Q-9 system (192). Since then, several authors have reported clear differences among *T. beigelii* isolates based on physiological, morphological, and genetic characteristics.

In 1991, Kemker et al. started to describe clearly the diversity among *Trichosporon* spp. isolated from environmental and clinical sources (94). These authors evaluated different isoenzymes present in 15 *Trichosporon* strains and the genetic profiles of these strains by using restriction fragment length polymorphisms (RFLP) analysis of ribosomal DNA (rDNA), including both the internal transcribed spacer 1 (ITS1) and ITS2 regions and the 5.8S gene as targets. These data were

compared with morphological features, carbon substrate usage profiles, and uric acid assimilation abilities.

In 1992, Gueho and collaborators conducted molecular studies of nucleic acids correlated with the morphological, physiological and biochemical characteristics of a collection of strains and proposed the reassignment of the genus *Trichosporon* (69). The criteria used to reclassify *Trichosporon* species were based on several aspects, including the ultrastructure of septal pores. These authors demonstrated that although all *Trichosporon* species contain an electron-dense and multilamellar cell wall, at least two basic types of dolipores exist. The first is composed of nailhead-like swellings separated by a narrow pore canal that is often filled by an electron-dense mass, whereas the second is constituted by well-defined, rounded inflations with a sponge-like or vesicular substructure covered by a parentheses. Other characteristics, such as coenzyme Q systems Q-10 and Q-9, differed within *Trichosporon* spp. and corresponded to the guanine-plus-cytosine content (moles percent G+C). DNA reassociation values, nutritional profiles, and evaluation of part of the 28S regions of rDNA sequences also demonstrated differences between *Trichosporon* species. Following analysis of 101 strains, these authors described a new species (*Trichosporon mucooides*) and 19 taxa; additionally, some previously described species were designated synonyms of other species.

In the same year, Gueho et al. reported on the variety of the genus and suggested that various series of species can be recognized based upon different niches (66). Strains differ based on their source of isolation (soil, water, or human or animal disease) and based on their phenotypic and molecular properties. The two species *T. beigelii* and *T. cutaneum*, considered to be synonyms since 1942, were clearly different as determined by ubiquinone systems and percent G+C DNA content. Therefore, the authors considered the nomenclature *T. beigelii* to be inappropriate and suggested that it be abandoned. In 1994, those authors concluded that the genus *Trichosporon* should include the following six species: *T. cutaneum*, *T. asahii*, *T. asteroides*, *T. mucooides*, *T. inkin* and *T. ovoides* (67).

Therefore, the previous taxon *T. beigelii* was replaced by several species, and the taxonomy of the genus was progressively modified by powerful molecular tools able to discriminate between phylogenetically closely related species (66, 176, 177). In 1994 and 1995, Sugita et al. reviewed the genus *Trichosporon* and proposed a new classification including 17 species and 5 varieties of *Trichosporon* (176, 177). In 2002, Sugita et al. proposed 25 species for the genus *Trichosporon* and suggested that 8 should be considered relevant as potential human pathogens, including the two emergent species *T. domesticum* and *T. montevidense* (172). Thereafter, the same group published a report in 2004 recognizing 36 *Trichosporon* species, including 5 new species proposed by Middlehoven et al. in 2004: *T. vadense*, *T. smithiae*, *T. dehoogii*, *T. scarabaeorum*, and *T. gamsii* (169). In 2004, Middlehoven et al. also separated the order Trichosporonales into four clades named Gracile, Porosum, Cutaneum, and Ovoides (Table 1) (123). In that same year, Sugita et al. (169) included the clade Brassicae in the order Trichosporonales, which encompassed some species considered to belong to the Gracile clade by Middlehoven et al. (123) (Table 1).

A new *Trichosporon* species isolated from the hindgut of the

TABLE 1. Currently accepted *Trichosporon* species and their subdivision within different clades

Clade	Species no.	Species name <sup>a</sup>
Gracile/Brassicaceae	1	<i>Trichosporon brassicae</i>
	2	<b><i>Trichosporon domesticum</i></b>
	3	<b><i>Trichosporon montevidense</i></b>
	4	<i>Trichosporon scarabaeorum</i>
	5	<i>Trichosporon mycotoxinivorans</i>
	6	<i>Trichosporon dulciturum</i>
	7	<i>Trichosporon cacaoliposimilis</i>
	8	<i>Trichosporon gracile</i>
	9	<i>Trichosporon laibachii</i>
	10	<i>Trichosporon multisporum</i>
	11	<i>Trichosporon vadense</i>
	12	<i>Trichosporon veenhuisii</i>
	13	<i>Trichosporon akiyoshidainum</i>
	14	<i>Trichosporon chiropterorum</i>
	15	<i>Trichosporon siamense</i>
	16	<i>Trichosporon otae</i>
	17	<i>Trichosporon loubieri</i>
Cutaneum	18	<b><i>Trichosporon cutaneum</i></b>
	19	<i>Trichosporon debeurmannianum</i>
	20	<b><i>Trichosporon dermatis</i></b>
	21	<b><i>Trichosporon jirovecii</i></b>
	22	<i>Trichosporon oleaginosus</i>
	23	<i>Trichosporon moniliiforme</i>
	24	<b><i>Trichosporon mucoides</i></b>
	25	<i>Trichosporon smithiae</i>
	26	<i>Trichosporon terricola</i>
	27	<i>Trichosporon middelhovenii</i>
	28	<i>Trichosporon shinodae</i>
	29	<i>Trichosporon cavernicola</i>
Porosum	30	<i>Trichosporon aquatile</i>
	31	<b><i>Trichosporon asahii</i></b>
	32	<b><i>Trichosporon asteroides</i></b>
	33	<i>Trichosporon caseorum</i>
	34	<b><i>Trichosporon coremiiforme</i></b>
	35	<b><i>Trichosporon faecale</i></b>
	36	<b><i>Trichosporon inkin</i></b>
	37	<b><i>Trichosporon japonicum</i></b>
	38	<b><i>Trichosporon lactis</i></b>
	39	<b><i>Trichosporon ovooides</i></b>
	40	<i>Trichosporon insectorum</i>
	41	<i>Trichosporon porosum</i>
	42	<b><i>Trichosporon dohaense</i></b>
	43	<i>Trichosporon chiarelli<sup>b</sup></i>
	44	<i>Trichosporon xylopinii</i>
Ovooides	45	<i>Trichosporon dehoogii</i>
	46	<i>Trichosporon gamsii</i>
	47	<i>Trichosporon guehoae</i>
	48	<i>Trichosporon lignicola</i>
	49	<i>Trichosporon sporotrichoides</i>
	50	<i>Trichosporon wieringae</i>

<sup>a</sup> The species in bold were described as being of medical interest.

<sup>b</sup> Closely related but placed in a different clade by Pagnocca et al. (139).

lower termite *Mastotermes darwiniensis*, recognized as an important detoxifier of mycotoxins, was described by Molnar et al. in 2004 and named *T. mycotoxinivorans*, belonging to the clade Gracile (125).

In 2005, a study addressing the isolation and identification of yeasts from guano of bat-inhabited caves in Japan proposed seven novel potential *Trichosporon* species. The new species were not representative of a single clade (Gracile, Porosum,

Brassicaceae, Cutaneum, or Ovooides) but were distributed across all but the Ovooides clade (170). In 2008, Fuentefria proposed a new *Trichosporon* species isolated from the guts of insects from Panama and from artesian cheese prepared in Brazil. The new species was included in the Ovooides clade (60).

In 2009, a new *Trichosporon* species was described in Qatar. Taj-Aldeen et al. described a novel *Trichosporon* species named *T. dohaense* following the investigation of 27 clinical isolates of *Trichosporon* obtained from immunosuppressed patients. Three isolates of the new species were cultured from patients with onychomycosis, catheter-related infection, and tinea pedis. The novel species is closely related to *T. coremiiforme* and belongs to the Ovooides clade (180).

In 2010, in Brazil, a new *Trichosporon* species was described based on the analysis of the D1/D2 and ITS regions of 34 *Trichosporon* strains closely related to *T. scarabaeorum*. The new species was named *T. chiarelli*, and strains were isolated from a fungus garden and from waste. This novel species was considered a sibling species of the Cutaneum clade (139).

Recently, other *Trichosporon* species have been described. The first species was originally cultured from the gut of the wood-resident beetle *Xylopinus saperdioides* and named *T. xylopinii*. It is clearly distinguishable from its closest relative, *T. porosum*, by 14 nucleotides in the ITS and D1/D2 regions of the rRNA genes. Of note, *T. xylopinii* has been recognized as an arthroconidium-producing yeast that is able to degrade hemi-cellulose (71). Two other novel *Trichosporon* species were characterized based on the analysis of oleaginous yeasts maintained at the American Type Culture Collection (ATCC Mycology Collection). They were named *T. cacaoliposimilis* and *T. oleaginosus*, respectively. Molecular phylogenetic analyses showed that *T. cacaoliposimilis* belongs to the Gracile clade, close to *T. gracile* and *T. dulciturum*, whereas *T. oleaginosus* belongs to the Cutaneum clade, with *T. jirovecii* as the closest taxon (70).

In conclusion, as illustrated in Table 1, 50 species of *Trichosporon* have been described from different regions of the globe, including 16 species with clinical relevance (139). The previously recognized taxon *T. pullulans*, which was considered to belong to the genus *Trichosporon* by Diddens and Lodder in 1942, has now been reassigned to a new genus and is named *Guehomyces pullulans* (51).

Based on the large variability of natural habitats related to all different species of *Trichosporon*, it is possible to suggest that different reservoirs may play a role in human infections.

#### VIRULENCE FACTORS OF *TRICHOSPORON* SPP. AND SOURCES OF INVASIVE INFECTIONS

Fungi that are opportunistic pathogens retain several factors that allow their growth and permit the establishment of disease and their dissemination within the host. These factors are known as virulence factors. Such factors are usually related to morphological switching, the ability to adhere to abiotic surfaces, thermotolerance, the expression of cell wall components, and enzyme production and secretion (77, 87, 145). Even though *Trichosporon* spp. can represent the second or third most common non-*Candida* yeast infections causing invasive disease in patients with hematological cancer, few reports have addressed the virulence factors of this genus (24, 42, 138).

### Biofilms

Infections with invasive *Trichosporon* spp. are usually associated with central venous catheters, vesical catheters, and peritoneal catheter-related devices. The ability to adhere to and form biofilms on implanted devices can account for the progress of invasive trichosporonosis, as it can promote the escape from antifungal drugs and host immune responses. Di Bonaventura et al. were the first to use electron microscopy to analyze the kinetics of biofilm formation and development on polystyrene surfaces. They tested 4 *T. asahii* strains, 3 of which were isolated from blood samples of patients with hematological malignancies (42). Those authors showed that, similarly to data described for biofilms of *Candida* spp., *T. asahii* cells were able to rapidly adhere to polystyrene after a 30-min incubation. These cells presented different morphologies, such as budding yeasts and filamentous forms, embedded within an extracellular polysaccharide (EPS) matrix composing the ultrastructure of the mature biofilm after 72 h. Analysis of confocal imaging sections taken across the biofilm showed that the thickness of mature biofilms varied from 25 to 40  $\mu\text{m}$ . These authors also tested the susceptibility profiles of planktonic and biofilm cells to amphotericin B, caspofungin, fluconazole, and voriconazole. *T. asahii* biofilms were resistant to all antifungals tested (MIC > 1,024  $\mu\text{g/ml}$ ) and were up to 16,000 times more resistant to voriconazole than planktonic cells (MIC = 0.06  $\mu\text{g/ml}$ ).

### Enzymes

Another important virulence factor is the ability to produce and secrete enzymes for scavenging nutrients from the environment. Proteases and phospholipases are among the enzymes that can increase fungal pathogenicity by breaking up proteins and disrupting host cell membranes, depending on their expression levels and the host immune response (61).

In 1994, Chen and collaborators purified two lipase enzymes (lipases I and II) from *T. fermentans* WU-C12 and verified that these enzymes could hydrolyze olive oil with an optimum pH of 5.5 and temperature of 35°C (31). These lipases also demonstrated stable activity after incubation at 30°C for 24 h over a pH range of 4.0 to 8.0 and were further characterized by cDNA isolation and sequencing (7). BLAST (Basic Local Alignment Search Tool, <http://blast.ncbi.nlm.nih.gov/>) analysis showed that the cDNA encoded by the gene *TFL1* and the putative protein sequence had 99.5% similarity to those of lipase II from *Geotrichum candidum*. Southern blot analysis revealed that there were two lipase genes carried on the genome, confirming the prior purification of two lipases. A putative correlation between the ability to produce Tfl1 lipase and the virulence of *Trichosporon* in humans has not been examined. However, one can speculate that lipases in general are important for the invasion process and for adaptation to different tissues within the host.

With regard to *Trichosporon* spp. isolated from infected patients, Chaves and collaborators have analyzed the proteinase and phospholipase activities of a collection of 30 yeast isolates, including two strains of *T. pullulans* (30). Both of the *T. pullulans* isolates did not exhibit phospholipase activity but did display proteinase activity. This result is reasonable in light of

the reclassification of *T. pullulans* as *Guehomyces pullulans*, which could be a phospholipase nonproducer.

Dag and Cerikçioğlu analyzed the production of a variety of virulence factors from 50 *T. asahii* strains, the majority of which were isolated from urine, although some originated each from blood, peritoneal fluid, nephrostomy, tongue swab, and nail. Proteinase and phospholipase activities could not be detected from these strains, although all isolates were esterase positive. Slime production was moderate in 10 strains, while 18 strains were characterized as weak producers and 20 strains were defined as slime nonproducers (37).

Later, Cafarchia and collaborators investigated the phospholipase activity in a collection of 163 yeast isolates recovered from pigeon cloacae and droppings (22). Biochemical and morphological tests identified 13 species among these isolates, of which 14 isolates were identified as *T. beigeli*. Two of the 10 isolates recovered from cloacae exhibited phospholipase activity in egg yolk medium plates after 2 and 5 days of incubation, whereas all 4 isolates from excreta exhibited phospholipase activity. This was the first report showing phospholipase activity in *Trichosporon* spp., and no gene in the genome has been described to have this function.

Recently, an alkaline lipase was isolated from *T. asahii* MSR 54, which was previously cultured from petroleum sludge in India for application in developing a presoak formulation for oil removal (100). The enzyme was active at ambient temperature but presented maximal activity at 40°C and over a pH range of 8.0 to 10.0. An analysis of the production of extracellular enzymes and the morphology switch of 61 clinical isolates of *T. asahii* revealed that these isolates were not able to produce secreted aspartic proteinases or phospholipases, while they were able to secrete active beta-*N*-acetylhexosaminidase. No other clinically relevant *Trichosporon* spp. have been shown to produce this enzyme (84). The same study also reported 4 different morphological types of colonies grown on Sabouraud dextrose agar (SDA), i.e., white farinose (69%), white pustular (18%), yellowish white (10%), and white cerebriform (3%). Those authors observed that strains of the three major types usually developed two to five colony types and switched at a frequency of 10<sup>2</sup> to 10<sup>4</sup> when grown at 37°C, similar to that observed with *Candida albicans* and *Cryptococcus neoformans* (4, 59, 87). Most of the colonies switched irreversibly to the smooth type, which had the greatest beta-*N*-acetylhexosaminidase enzymatic activity compared with the parent type in all strains (84).

Despite the available information on the capability of *Trichosporon* strains to produce lipases and proteases, the roles of these enzymes in the pathogenesis of human invasive infections are not clear. In addition, this genus may possess cryptic enzymes not yet described.

### Cell Wall Components

Members of *Trichosporon* spp. express glucuronoxylomanan (GXM) in their cell walls, similarly to *C. neoformans*. GXM is a 1,3-linked mannan backbone attached to short side chains of 1,4-linked mannose and 1,2-linked xylose residues by substituting the 2 or 4 portion of the 1,3-linked mannose residues of the main group (124). This polysaccharide may attenuate the phagocytic capability of neutrophils and monocytes *in*

*vivo* (150). Karashima and collaborators reported that *T. asahii* isolates could switch phenotype and increase the amount of secreted GXM after passage in a mouse model (91). Three environmental isolates were analyzed before and after consecutive passages through mice and compared with 14 isolates from patients with deep-seated infections or recovered from lung autopsies, blood, urine, sputum, and catheters. That study demonstrated that the colonies of all of the environmental isolates were rugose, whereas the majority of the clinical isolates were powdery when grown on SDA. The clinical isolates consisted of 90% blastoconidia and arthroconidia, whereas all of the environmental isolates consisted of 99% hyphae. Interestingly, all environmental isolates from mouse kidneys recovered in SDA switched their macromorphologies to powdery colonies, with conidia as the predominant cell type. All clinical isolates released significantly higher levels of GXM than did environmental isolates (titers of  $\log_2 9.4 \pm 0.7$  versus  $\log_2 5.4 \pm 1.4$ ). After passage in mice, environmental isolates generated increased GXM concentrations (titers of  $\log_2 10.0 \pm 0.7$  versus  $\log_2 5.4 \pm 1.4$ ). Minor changes in the (1 $\rightarrow$ 3)- $\beta$ -D-glucan (BDG) titers were observed, and no significant difference in titers was detected for environmental isolates before and after passage in mice. Taken together, the changes in the phenotype of the environmental isolates after passages in mice seem to be a regular response to adaptation within the host in order to facilitate dissemination and escape from phagocytosis by polymorphonuclear leukocytes and monocytes *in vivo*.

The protective role of GXM was also addressed by analyzing the structure and function of GXM isolated from *T. asahii* and testing whether this polysaccharide could protect acapsular *C. neoformans* mutants from phagocytosis by mouse macrophages. Fonseca and collaborators reported that the concentration of GXM in the supernatants of *T. asahii* cultures was approximately 12 times less than that in *C. neoformans* supernatants (56). The major components of *T. asahii* GXM were mannose (60%), followed by xylose (24%) and glucose (8%). This differed from the case for *C. neoformans* supernatants, from which the third most commonly isolated component was glucuronic acid (10%). Fluorescence microscopy also demonstrated that *T. asahii* cells possessed less GXM in their cell walls than *C. neoformans* cells. To test the protective effect of GXM against phagocytosis, acapsular *C. neoformans* mutant cells were coated with GXM purified from a *C. neoformans* high-GXM producer and with GXM from *T. asahii* and incubated with murine macrophage cells *in vitro*. Yeast cells containing no detectable surface GXM were rapidly internalized by phagocytes, and the polysaccharide-coated cells were phagocytized 6 times less frequently. However, no significant difference was observed with regard to the GXM source, indicating that despite differences in major sugar components, GXM from *T. asahii* had a protective role similar to that of GXM from *C. neoformans* (56, 84).

#### Sources of Superficial and Invasive Infections

The source of superficial and deep-seated *Trichosporon* infections is still the subject of considerable debate. It is important to consider that *Trichosporon* spp. are widely distributed in nature and may be part of the normal biota of the skin, respiratory tract, gastrointestinal tract, and vagina (24, 187).

The mode of transmission of superficial trichosporonosis remains unclear, but poor hygiene habits, bathing in contaminated water, and sexual transmission may play a role (17, 96). Although there is no consensus on the route of infection of human beings, close contact with an incident case, hair humidity, and the length of the scalp hair have all been recognized as risk factors for acquiring white piedra (96, 153, 158, 197).

In regard to deep-seated infections, gastrointestinal colonization and further translocation throughout the gut may be considered the source of infection in a considerable number of episodes of trichosporonosis documented in cancer patients. In support of an endogenously acquired route of infection, we may look to evidence in support of gut translocation as the major source of candidemia, a biological model that may explain invasive infections caused by yeast pathogens that colonize the gastrointestinal tract (34, 134). In developing the first animal model of invasive trichosporonosis, Walsh and colleagues showed that *Trichosporon* was able to disseminate from the gut to the blood and other organs in rabbits after immunosuppression but not in healthy animals (189).

In addition to endogenous acquisition from the gut, evidence also suggests that a substantial number of trichosporonosis cases may be exogenously acquired. *Trichosporon* spp. may enter the blood after contamination of a percutaneously inserted intravascular catheter via colonized skin. Indeed, Kontoyiannis et al. evaluated 17 cancer patients with trichosporonosis and suggested that central venous catheter-related fungemia was the cause of 70% of all episodes (98). Interestingly, those authors suggested that prophylaxis with fluconazole, frequently used in patients with hematological disease, may prevent the gut translocation of *Trichosporon* spp. in this population, making the exogenous acquisition of this pathogen (i.e., mediated by colonization of a central venous catheter) the most plausible source of infection in this specific population.

Finally, infants with very low birth weight (VLBW) may develop trichosporonosis, despite it being a rare cause of sepsis in neonates. Either yeast gastrointestinal translocation or catheter-associated fungemia may occur in these patients. Neonate skin colonization by *Trichosporon* may be acquired either from health care workers or after vaginal delivery, as up to 14% of woman may harbor this organism in their vulvovaginal region (45, 156).

#### HUMAN INFECTIONS CAUSED BY MEMBERS OF THE GENUS *TRICHOSPORON*: AT-RISK POPULATIONS AND CLINICAL MANIFESTATIONS

*Trichosporon* is a medically important genus whose members are able to colonize and proliferate in different parts of human body, including the gastrointestinal system, respiratory tract, skin, and vagina. This yeast-like pathogen may cause deep-seated, mucosa-associated, or superficial infections. Invasive trichosporonosis is documented mostly in patients with hematological malignancies and other medical conditions associated with immunosuppression, whereas superficial infections and allergic pneumonia are found predominantly in immunocompetent hosts (24, 46, 98, 184). Table 2 summarizes the sources of infection, main associated conditions, and etiological agents

TABLE 2. Human trichosporonosis: sources of infection, main associated conditions, and etiological agents

Infection category	Main type(s) of infection	Major agent(s)	Main associated conditions
Invasive	Fungemia, urinary tract infections, peritonitis, endocarditis, others <sup>a</sup>	<i>T. asahii</i> , <i>T. mucoides</i> , <i>T. asteroides</i>	Cancer, vascular and urinary catheters, organ transplantation, broad-spectrum antibiotic therapy
Allergic pneumonia	Summer-type hypersensitivity pneumonitis	<i>T. cutaneum</i> <sup>b</sup>	Hot and humid weather, environmental contamination
Superficial	White piedra	<i>T. inkin</i> , <i>T. cutaneum</i> , <i>T. ovoides</i> , <i>T. loubieri</i>	Young age and female sex, long hair, humidity, poor hygiene, headband use

<sup>a</sup> Arthritis, esophagitis, meningitis, brain abscess, splenic abscess, and uterine infections.

<sup>b</sup> *T. cutaneum* is now considered a complex of 10 different species.

that are involved with the most clinically relevant *Trichosporon* infections.

### Superficial Infections

The clinical presentation of *Trichosporon* infection in humans is often benign superficial lesions of hair, called white piedra, characterized by the presence of irregular nodules on the affected hair. These nodules are loosely attached to the hair shaft, have a soft texture, and may be white or light brown. White piedra is a cosmopolitan disease affecting children and adults from areas with tropical and temperate climates. Most cases of white piedra have been reported in children and young adults, particularly females who frequently use headbands. Although rarely reported in the United States, this disease may be found in Texas and may be underreported in several regions (63, 96, 159).

White piedra may be found in a large variety of hairy regions, including the scalp, beard, moustache, eyebrows, axilla, and, particularly, genital hairs. Concomitant hair infection by *Trichosporon* spp. and corynebacteria has been demonstrated by electron microscopy and histochemical evaluation of concretions, and it is still unclear whether the proteolytic capabilities of the coryneform bacteria may be relevant for the establishment of fungal infection (53, 54, 65, 67, 92, 96, 177, 197).

White piedra appears to be caused predominantly by *T. inkin*, *T. cutaneum*, *T. ovoides*, and *T. loubieri*, which has recently been reported in the literature as an emergent species related mainly to superficial infections in humans (9, 67, 99, 137, 177). It is noteworthy that *Trichosporon* spp. can also cause other superficial infections, such as onychomycosis, where the more frequently isolated species is *T. cutaneum*. Indeed, some Mexican authors have documented that the isolation of *Trichosporon* spp. from tinea pedis and onychomycosis patients may range from 2.81% to 42.8% of cases (9, 121, 155).

### Deep-Seated Infections

After the first case of invasive trichosporonosis described by Watson and Kallichurum in 1970 (190a), several cases of invasive trichosporonosis in different clinical scenarios were described. For instance, in 1982 Manzella et al. reported a case of fungemia with cutaneous dissemination due to a *Trichosporon* sp. documented in a leukemic patient followed until death (113). Some years later, in 1988, Reinhart et al. reported a case of endocarditis caused by a *Trichosporon* sp. in a patient who

had previously suffered from rheumatic disease (148). In 1997, Lopes et al. described a case of peritonitis due to *T. inkin* in a diabetic patient (107). In 2001, Moretti-Branchini et al. reported 2 cases of invasive *Trichosporon* infections involving 2 patients with bone marrow transplants hospitalized at the Clinical Hospital of The University of Campinas, Brazil (126). A case of chronic dissemination infection due to a *Trichosporon* sp. resulting in multiple liver abscesses was reported by Meyer et al. in 2002 (122). In 2005, Abdala et al. reported a case of invasive *T. asahii* infection in a nonneutropenic patient undergoing orthotopic liver transplantation who died despite treatment with amphotericin B (1).

After combining all of the reported cases of trichosporonosis, one can suggest that *Trichosporon* spp. appear to be increasing agents of invasive mycoses in contemporary medicine. In patients with malignant hematological diseases, this genus has been reported as the second most common agent of disseminated yeast infections, behind only the genus *Candida*, leading to 50 to 80% mortality rates despite treatment with antifungal therapy (55, 99, 138, 188). Indeed, breakthrough trichosporonosis in immunocompromised patients after administration of amphotericin B and echinocandins, and more rarely after the use of triazoles, has been extensively reported (15, 64, 79, 118, 186).

The largest retrospective multicenter study of invasive trichosporonosis and geotrichosis in patients with malignant hematological diseases was conducted by Girmenia et al. in 2005 and included data on *Trichosporon* and *Geotrichum* infections documented over 20 years (62). Those authors reviewed 287 cases of trichosporonosis and 99 cases of geotrichosis documented from all over the world. The most common underlying conditions related to trichosporonosis were hematological diseases, peritoneal dialysis, and solid tumors. Trichosporonemia occurred in 115/154 (74.7%) of patients and disseminated infection in 78/154 (50.6%) of cases. The majority of the cases of trichosporonosis and geotrichosis were reported in North American medical centers (33.9%), followed by medical centers from Europe (27.6%) and Asia (23.3%). Only 6 isolates from South American institutions were reported, including 5 Brazilian isolates and 1 isolate from Argentina. Despite the large number of *Trichosporon* isolates included in this review, two were accurately identified to the species level as *T. loubieri* and 8 were assigned to the old taxon *T. pullulans* (recently named *G. pullulans*), while the other 287 isolates were identified only as *Trichosporon* sp.

In 2004, Kontoyannis et al. described the clinical spectrum and outcome of 17 patients with cancer and invasive tricho-

sporosis documented at the MD Anderson Cancer Center. The overall incidence of invasive trichosporosis was found to be 8 cases per 100,000 admitted patients; 65% of the infected patients had acute leukemia, and 65% had neutropenia. Most patients (59%) had fungemia as the sole manifestation of the fungal infection, and 7 of 10 with *Trichosporon* fungemia had a central venous catheter-related infection. Of note, 60% of episodes were documented in patients who had been exposed to at least 7 days of antifungal therapy (breakthrough infections). The crude mortality rate at 30 days after admission was 53% (98).

In 2009, Ruan et al. described a series of 19 patients with invasive trichosporosis documented between 2000 and 2008 at the National Taiwan University Hospital. Cancer was the underlying disease in 58% of patients, and only 4 patients (21%) were neutropenic at the time of the diagnosis. Central venous catheter placement and the use of antibiotics were the most commonly associated conditions, being present in 90% and 95% of all patients, respectively. The crude mortality rate at 30 days after infection was 42% (154).

In 2010, Suzuki et al. retrospectively evaluated clinical aspects and outcomes for 33 patients with *Trichosporon* fungemia and hematological malignancies in 5 different Japanese tertiary care centers between 1992 and 2007. The majority of these patients had acute leukemia (82%) and neutropenia (85%), and 90% of them had been exposed to at least 5 days of systemic antifungal therapy (breakthrough infections). Skin lesions were reported in 12 patients and pneumonia in 19 patients. The mortality rate attributable to the fungus was found to be 76%, with 67% of deaths occurring within 10 days of admission (179).

Critically ill patients admitted to intensive care units and subjected to invasive medical procedures and antibiotic treatment are the patients most commonly associated with trichosporosis, other than patients with malignant diseases (24, 25, 44, 154, 191). A recent paper from Chagas-Neto et al. (25) showed that in a series of 22 Brazilian pediatric and adult patients with trichosporosis fungemia, most were ICU patients with no malignant disease. Most infected patients had degenerative diseases with organ failures and were subjected to treatment with broad-spectrum antibiotics and multiple invasive medical procedures before developing fungemia. Crude mortality rates were 30% for children and 87.5% for adult patients ( $P = 0.025$ ) (24, 25). Finally, small series of cases of invasive trichosporosis have also been described for burn victims, patients with secondary hemochromatosis, and Job's syndrome (23, 26, 73, 162).

Based on these series of cases, it is possible that fungemia and fever represent the most common clinical findings described for *Trichosporon* hematogenic dissemination. However, despite being less frequent, there are increasing reports of episodes of organ-specific infections and patients with disseminated trichosporosis presenting with pneumonia, soft tissue lesions and eventually endophthalmitis, endocarditis, brain abscess, meningitis, arthritis, esophagitis, lymphadenopathy, liver, splenic abscess, and uterine infections (23, 24, 26, 27, 74, 98, 110, 117, 154, 178).

Peritonitis is one of the most common and serious complications of peritoneal dialysis, and a case of peritoneal infection due to *Trichosporon* spp. in this setting was documented by

Khanna et al. in 1980 (95). Fungal peritonitis is usually documented in patients with a history of previous bacterial infections, and this condition presents with sign and symptoms usually associated with any microorganism causing dialysis-associated peritonitis: fever, abdominal fullness, and turbid peritoneal dialysate fluid (88, 111).

Despite being considered rare, endocarditis due to *Trichosporon* species in native or prosthetic valves of nonneutropenic patients has been increasingly reported. Patients usually develop large vegetations that may lead to embolic phenomena frequently located on the aortic bifurcation, in arteries of the lower extremities, or eventually in the brain. As suggested by most reports, valve replacement is mandatory, but recurrence of infection is very common and prognosis is generally poor regardless of the antifungal therapy (29, 86, 93, 114, 116, 147–149, 164, 182).

Urinary tract infections by this pathogen may also occur, especially in patients with urinary obstruction or those undergoing vesical catheterization and antibiotic treatment. These infections represent a clinical challenge for clinicians, as there are no clear and specific indications for the clinical interpretation of *Trichosporon* sp. recovery in urine. Although unusual, renal damage and aggravation of renal dysfunction may occur (50, 127, 191).

Although most published reports on *Trichosporon* disseminated infections present scarce data in terms of species identification of the etiological agents using molecular methods, *T. asahii* is the most frequently isolated species in these infections (25, 154, 179).

Finally, Ando et al. have described that the genus *Trichosporon*, in addition to causing infections, is the major etiological agent of summer-type hypersensitivity pneumonitis (SHP) in Japan (5). The authors investigated 621 patients with SHP and found that 95% of them had anti-*Trichosporon* antibodies in serum. *Trichosporon* isolates were found extensively in patients' houses, and the elimination of these organisms from patients' houses prevented disease (6, 163, 195, 196). *Trichosporon* species are responsible for SHP, leading to type III and IV allergies via repeated inhalation of organic dust and fungal arthroconidia that contaminate home environments during the hot, humid, and rainy summer season in western and southern Japan (11, 65, 92, 133, 169, 181, 188, 194). SHP is an immunologically induced lung disease, with pathogenesis mechanisms involving an initial immune complex-mediated lung injury followed by cell-mediated tissue damage with massive lymphocyte infiltration into the lungs (90). Mizobe et al. have characterized the antigenic components involved in SHP as GXM (124).

*T. cutaneum* was originally described as the major agent of SHP, exhibiting 4 different genotypes: I, II, III, and I-III. After the extensive revision of the taxonomy of *Trichosporon* spp., the taxon *T. cutaneum* was divided into more than 10 different species, which tend to be grouped within the previously described serotypes (6, 85, 133, 169).

#### DIAGNOSIS OF INVASIVE TRICHOSPORONOSIS AND LABORATORY TOOLS FOR IDENTIFICATION OF *TRICHOSPORON* SPP.

In accordance with the definitions of opportunistic invasive fungal infections (IFI) published by the European Organization for Research and Treatment of Cancer/Invasive Fungal

Infection Cooperative Group (EORTC/IFICG) and the National Institute of Allergy and Infectious Disease Mycoses Study Group (NIAID/MSG), invasive trichosporonosis may be defined as follows (40).

1. "Proven invasive trichosporonosis." Patients with proven invasive trichosporonosis presenting at least one of the following criteria: (i) blood cultures yielding *Trichosporon* species in patients with temporally related clinical signs and symptoms of infection, (ii) cerebrospinal fluid (CSF) cultures yielding *Trichosporon* species, or (iii) biopsy specimens that are culture positive and present histopathological evidence of fungal elements compatible with *Trichosporon* spp.
2. "Probable invasive trichosporonosis." Patients with probable invasive trichosporonosis present all of the following criteria: (i) presence of at least one host factor (therapy with an immunosuppressive drug[s], neutropenia, or persisting fever despite therapy with appropriate broad-spectrum antibiotics), (ii) one microbiological criterion (culture or presence of fungal elements compatible with *Trichosporon* in a suspect biological material), and (iii) one major clinical criterion (imaging or cytochemical findings) consistent with infection.

As the EORTC/IFICG and NIAID/MSG definitions do not provide specific indications for the clinical interpretation of *Trichosporon* sp. recovery from respiratory tract specimens, we have adapted the criteria for diagnosis of "probable" pulmonary infection proposed by Girmenia and collaborators (62). These criteria require the following evidence for diagnosis of probable pneumonia: the presence of pulmonary infiltrates and recovery of *Trichosporon* species from sputum or bronchoalveolar lavage (BAL) fluid samples in the absence of other pathogens causing opportunistic infections.

Microbiological diagnosis of superficial and invasive trichosporonosis classically relies on culture findings as well as the identification of fungal elements compatible with *Trichosporon* spp. (hyphae, pseudohyphae, arthroconidia, and blastoconidia) in wet mount and/or tissue biopsy specimens. Although demonstration of fungal organisms in tissue and cultures is considered the "gold standard" for defining invasive infections, obtaining biopsy specimens may be difficult for some patients. Consequently, nonculture methods for the diagnosis of invasive mycosis are increasingly needed to surpass the limitations of test sensitivity, delayed results, and problems in distinguishing between colonized and truly infected patients.

### Antigen Detection

The detection of (1 $\rightarrow$ 3)- $\beta$ -D-glucan (BDG) has been used extensively for early diagnosis of invasive fungal infections, including candidiasis and aspergillosis (136). There are no reports in the English language literature specifically addressing the sensitivity and specificity of BDG measurement in the diagnosis of invasive trichosporonosis. However, a recent publication reported epidemiological and clinical aspects related to 33 cases of *Trichosporon* fungemia in Japan (179). In that study, only 50% of patients had a single test positive for BDG at the time of admission, and few had an antigen-positive test

prior to their positive blood cultures. Therefore, based on the experience of this series of cases, it appears that the measurement of BDG levels may have serious limitations for the early diagnosis of invasive trichosporonosis.

It is well known that *Trichosporon* strains have GXM in their cell wall, which can lead to a cross-reaction with *C. neoformans* antigens in sera of patients with invasive trichosporonosis (120). According to Lyman and collaborators, all *Trichosporon* strains can produce measurable levels of GXM antigen, and thus the detection of anticryptococcal cross-reactive antigen may be an useful tool in the early diagnosis of *Trichosporon* infections (109). However, unlike for *C. neoformans*, the structural and serological properties of *Trichosporon* GXM have been poorly investigated (56). Therefore, considering the limited clinical information available on this topic, further clinical studies are necessary to confirm if the detection of *Trichosporon* GXM by using the *Cryptococcus* antigen test has potential for screening patients with invasive trichosporonosis.

### PCR-Based Methods

More recently, PCR-based methods and flow cytometry assays have been developed to diagnose invasive infection due to *Trichosporon* species. Though these new assays are not yet standardized to be used routinely in clinical settings, they represent important strategies for future clinical validation.

Nakajima et al. used a PCR technique to amplify the fungal ITS region. Total DNA was extracted from a lung biopsy specimen taken from a patient suffering from lung cancer. The ITS amplicon was sequenced, and this fragment identified *T. asahii* as the causative agent of the infection (131).

Fungal DNA can be extracted and detected not only from fresh tissues but also from paraffin-embedded tissue sections. Sano et al. (158) extracted DNAs from 30 different tissue sections obtained from major organs of 3 autopsy cases of disseminated trichosporonosis. The fungemic patients had been previously diagnosed by blood cultures and analysis with the Vitek 2 compact system to be infected by *T. asahii*. Pan-species and species-specific primer sets were used in nested PCRs to amplify different amplicon sizes of the rDNA region: 170 bp, 259 bp, and 412 bp. Out of 30 tissue samples tested, 20 were positive for *Trichosporon* sp. infection by hematoxylin and eosin (H&E) staining, and 22 were positive by detection using Grocott's stain. PCR amplification of the 170-bp fragment was positive in 20 of 22 samples, whereas only 12 of the 22 were positive for the 259-bp fragment. All of the 30 samples were PCR negative for the 412-bp fragment. Of the 8 samples that were negative by Grocott staining, only 1 sample was positive for both the 170- and 259-bp bands. The authors also verified that the efficiency of fungal detection by PCR decreased with longer periods of tissue fixation. After 1 day of fixation, only one-third of the collected samples were positive by both Grocott staining and amplification of the 170- and 259-bp bands. Longer tissue fixation of between 6 and 21 days considerably diminished the amplification of the 259-bp fragment (2 of 12) (158).

*Trichosporon* spp. can also be detected from biological fluids by using PCR assays. Nagai et al. developed a nested PCR assay to amplify part of the *T. asahii* 28S rDNA from a collection of 11 serum samples from patients with histologically diagnosed disseminated trichosporonosis. Of the 11 samples





FIG. 1. Schematic structure of the rRNA gene of *Trichosporon* spp. ITS, internal transcribed spacer; IGS, intergenic spacer region. The total length of the rDNA locus is approximately 7,850 bp, and the IGS1 region comprises 485 bp.

tested, 7 (64%) were PCR positive and 6 were positive for the GXM antigen (129).

Later, Sugita and collaborators used species-specific primers to amplify the rDNA region in nested PCRs for *T. asahii* diagnosis. A total of 11 serum samples from 7 different patients with deep-seated trichosporonosis confirmed after autopsy were tested by PCR assay and latex agglutination (LA) test for detection of *Trichosporon* spp. *T. asahii* DNA was detected by PCR from 9 of 11 samples, with only 7 samples also positive in the LA test (173).

Hosoki and collaborators (79) were able to detect breakthrough *Trichosporon* infection using serum PCR with a patient suffering from cytophagic histiocytic panniculitis and neutropenia. Those authors reported a study in which the patient's serum was screened every other week to PCR amplify bands related to fungal DNA. It was observed that until the third week after a cord blood transplant, the patient had no fungemia. On day 28, when the patient had been receiving prophylactic voriconazole for 17 days, *T. asahii* DNA was detected in blood samples, even though blood cultures remained negative. At that time, the patient had no symptoms, and BDG serum titers were normal. *T. asahii* DNA was detected by PCR in this patient's blood until day 39; the patient was then cured of the infection.

To improve the detection and identification of fungal infections in biological samples, high-throughput technologies, such as PCR with pan-species primer sets and DNA microarray, have been applied. Microarray assays were combined with multiplex PCR of unique ITS1, 18S, and 5.8S sequences and consecutive DNA microarray hybridization to diagnose 14 major fungal pathogens, including *T. asahii*. Ninety-one samples of blood, bronchoalveolar lavage fluid, and tissues were analyzed from 46 neutropenic patients representing 5 cases with no fungal infection and 5 cases with proven, 3 cases with probable, and 33 cases with possible IFI. In the single case of invasive trichosporonosis included in this series, *T. asahii* could be positively detected in blood and BAL fluid samples by the microarray assay (166).

Bottles of blood cultures collected from patients with invasive trichosporonosis have also been used to validate PCR assays as a tool for the diagnosis of fungemia and the further identification of the causative fungal species. Hsiue et al. developed an oligonucleotide array system targeting the ITS1 or ITS2 region to analyze 116 fungus-positive blood cultures. This system could identify 16 genera among 77 yeast species and was able to detect and identify 2 samples of *T. asahii* in blood cultures, compared to only one positive identification of *Trichosporon* spp. by phenotypic methods (82).

More recently, Nagano and collaborators compared culture-based methods with molecular techniques for the detection of fungi in 77 adult cystic fibrosis patients. Sputum samples were collected for DNA extraction, and the ITS region was ampli-

fied for sequencing analysis. *Trichosporon* spp. were identified in 2 of 77 samples by molecular techniques, whereas only one of those two samples was identified by culture methods (130).

### Luminex xMAP Technology

A novel flow cytometric technique called Luminex xMAP has been developed for the detection of medically important species of fungi. This assay is based on the use of PCR-biotinylated amplicon target DNA that is inoculated into a microsphere-bead mixture containing species-specific probes of interest. By adding a reporter molecule (streptavidin R-phycoerythrin), hybridized species-specific amplicons captured by complementary nucleotide sequences on microsphere beads can be recognized by the fluorescence of the reporter molecules.

Landlinger and collaborators (103) have used the Luminex xMAP technology to identify fungal species in samples from peripheral blood, blood cultures, pulmonary infiltrate biopsy specimens, bronchoalveolar lavage fluid, and bronchotracheal secretions and from isolated fungal strains. This system utilized 2 pan-*Trichosporon* probes designed to hybridize to the ITS2 region, which is highly variable among the genomes of individual fungal species, and it detected at least four clinically relevant *Trichosporon* species (*T. asahii*, *T. inkin*, *T. cutaneum*, and *T. beigeli*). Those authors reported that from one sample of paraffin-embedded tissue, a *Trichosporon* sp. was detected by specific hybridization signals, and following further sequencing analysis, this species was identified as *T. cutaneum*.

Not only is this technology useful for analysis of biological samples, it also has potential for the identification of *Trichosporon* spp. from culture isolates by using probes that recognize not only the ITS region and intergenic spacer 1 (IGS1) but also the D1/D2 region of the 28S rDNA (Fig. 1). Diaz et al. (41) used the Luminex 100 xMAP assay to identify 39 strains of different *Trichosporon* species. They showed that the ITS and D1/D2 regions were sufficient to discriminate between species and that the IGS region could differentiate closely related species such as *T. asahii*, *T. japonicum*, and *T. asteroides*.

### New Perspectives on Microscopic Imaging Diagnosis

Microscopic imaging analysis has also been improved in order to increase the ability to prove deep-seated *Trichosporon* spp. infections. Obana et al. (135) compared the performances of different histopathological stains for identifying fungal cells in tissues from 9 autopsy cases, including 3 cases of disseminated trichosporonosis, 3 cases of gastric candidiasis, 2 cases of pulmonary aspergillosis, and 1 case of pulmonary cryptococcosis. Those authors reported that *Trichosporon* cells (*T. asahii*) were weakly detectable within tissue sections compared with the ability to detect other fungal species when stained with conventional Grocott's stain. Using a mucicarmine stain, *Tri-*

*chosporon* could be weakly stained within the tissues, whereas *Candida* and *Aspergillus* were negatively stained. A colloidal iron stain, which reacts with *Cryptococcus* capsules, showed clear staining of *Trichosporon* cell walls as well as *Cryptococcus* capsules. A periodic acid-methenamine-silver stain (PAM) appeared to best discriminate fungal elements of *Trichosporon* spp. in tissue sections. Those authors used transmission electron microscopy to observe sections stained with Grocott's stain and diluted PAM to evaluate putative differences in cell wall silver deposition between *Candida* and *Trichosporon* strains. This strategy was useful for identifying differences in hyphal size as well as laminar deposition of silver granules in the cell wall. In conclusion, the authors suggested that histological staining procedures and electron microscopy may have potential as a tool for discriminating tissue infections by *Candida* and *Trichosporon* species.

### Phenotypic Identification of *Trichosporon* Species

The taxonomy of *Trichosporon* spp. has been completely rewritten, and the genus now includes 50 species (139), at least 16 of which have clinical relevance. This high number of new species presents a large variability in terms of virulence, clinical manifestations in humans, and antifungal susceptibility. Consequently, before defining strategies to treat and prevent superficial and fungal infections caused by *Trichosporon* spp., it is necessary to accurately identify the causative microorganism at the species level.

Several methods to identify *Trichosporon* species have been reported, including morphological and biochemical tests as well as molecular tools. Despite the fact that phenotypic methods are more suitable for routine use in general microbiology laboratories, the accuracy of the identification of *Trichosporon* spp. seems to be limited. Molecular methods are more precise in species identification but are costly for routine laboratory use (151, 172).

It is important to note that both *Trichosporon* and *Geotrichum* species are able to produce arthroconidia. In clinical laboratory analysis, when arthroconidia are visualized the urease test is recommended. Unlike *Geotrichum* spp., all species of the *Trichosporon* genus are able to hydrolyze urea (39). Although these genera are phenotypically similar, they are genetically very distinct. The ITS nucleotide sequences are less than 80% similar (33). Subsequently, specific diagnostic PCR can also be performed to differentiate these two genera (175).

Phenotypic methods for *Trichosporon* species identification are based on the characterization of micromorphological aspects of colonies as well as biochemical profiling. Performing a slide microculture to search for arthroconidia is a very useful tool for the screening of *Trichosporon* spp. However, other morphological aspects and biochemical tests do not allow the complete identification of *Trichosporon* isolates at the species level (151, 172). Indeed, by comparing the experiences of 3 different laboratories in evaluating the biochemical and physiological characteristics of 6 clinically relevant *Trichosporon* spp., significant discrepancies in the results generated by testing the same organisms were observed in different studies (24, 39, 144, 175). Therefore, one can conclude that classical methods for yeast identification have limited accuracy and reproducibility for *Trichosporon* spp. identification.

Despite limitations, several commercial nonautomated and automated systems have been used in the identification of *Trichosporon* spp. Notwithstanding the problems for biochemical tests mentioned above, it is also important to emphasize that many of these methods do not include new taxonomic categories in their databases. Consequently, the identification of the genus *Trichosporon* is oversimplified due to incomplete databases and classification keys.

The nonautomated commercial methods most commonly used for yeast identification in clinical laboratories are summarized below.

1. API 20C AUX (bioMérieux, Mercy l'Etoile, France). The API 20C AUX method is based on the evaluation of the ability of the fungus to assimilate 19 carbon sources. It requires additional tests, such as macro- and micromorphological observation of colonies and a urease production test. The results are read at 48 or 72 h. The current database includes only 3 species: *T. asahii*, *T. inkin*, and *T. mucoides* (48, 72, 144, 146).
2. ID 32C (bioMérieux, Mercy l'Etoile, France). The ID 32C method is based on the evaluation of the ability of the fungus to assimilate 24 carbon sources and 5 organic acids, evaluation of yeast sensitivity to cycloheximide, and esculin tests. It requires additional observation of the macro- and micromorphology of the colonies. This method shows inconsistent identification, and the database includes only *T. asahii*, *T. inkin*, and *T. mucoides* (18, 144, 146).
3. RapID Yeast Plus system (Innovative Diagnostic System, Norcross, GA). The RapID Yeast Plus system is based on the observation of 13 enzymatic hydrolysis substrates and assimilation of 5 carbon sources for the identification of clinically important yeasts (48). Kitch et al. (97) analyzed the ability of this technique to identify 304 clinical yeast isolates, 3 of which were *Trichosporon* spp., within 4 h. Two out of 3 isolates were identified exclusively as *Trichosporon beigelii*, though 1 isolate was misidentified as *C. neoformans*.

The automated systems for yeast identification most commonly used in clinical laboratories are summarized below. In general, all of the automated systems have severe limitations regarding the number of *Trichosporon* species listed in their databases.

1. Vitek Systems (bioMérieux, Vitek, Hazelwood, MO). The Vitek Systems system is based on nitrogen and carbon assimilation tests, enzymatic tests, and evaluation of yeast sensitivity to cycloheximide. The test readings are performed spectrophotometrically by the automated system after 24 and 48 h of incubation. The current database (Vitek 2) lists only *T. asahii*, *T. inkin*, and *T. mucoides*. Not only is this system limited by the number of species included in the database, but inconsistent results have been reported for the identification of *T. inkin* and *T. asteroides*, being erroneously identified as *T. asahii* by this system (2, 19, 43, 52, 83).
2. Baxter Microscan (Baxter Microscan, West Sacramento, CA). The Baxter Microscan method can generate final reports of yeast identification in 4 h by evaluating the

enzymatic profile of each organism tested. Fluorometric and spectrophotometric readings are taken and enriched by data generated by micro- and macromorphological observation of colonies. This system is considered to be less accurate than the Vitek system described above, and several authors have experienced difficulties in the correct identification of *Trichosporon* spp. using this method due to limitations in its database. To our knowledge, there has not been an update to the Microscan database since the original publications; therefore, the database still refers to all *Trichosporon* spp. as *T. beigeli* (102, 168).

Most papers addressing the accuracy of commercial yeast identification methods provide very little data on specific *Trichosporon* species. Moylett and collaborators claimed to identify 2 isolates of *Trichosporon pullulans* as the cause of chronic granulomatous disease by using the Vitek Yeast Biochemical card (128). At that time, this card version had only *T. beigeli* and *T. pullulans* in its database. To confirm their findings, Holland et al. examined the same two strains by amplification and sequencing of their ITS regions (78). BLAST analysis revealed that both yeasts initially identified phenotypically as *Trichosporon pullulans* were genotypically identified as *Cryptococcus adeliensis*. Ahmad and collaborators used the Vitek 2 system to identify a collection of clinical strains and found 29 isolates that they first identified as *T. asahii*. After all 29 identifications were checked by sequencing the ITS regions, 4 strains were reassigned as *T. asteroides* (2).

More recently, Leaw et al. (105) analyzed the accuracy of phenotypic tests and molecular markers (ITS1, ITS2, and 28S D1/D2 regions) for the identification of 373 medically relevant yeasts encompassing 86 species. A total of 74 strains were simultaneously identified by the API ID32C system and molecular methods. Of note, 27 *Trichosporon* strains representative of 9 different species (*T. aquatile*, *T. asahii*, *T. cutaneum*, *T. debrurmannianum*, *T. dermatis*, *T. inkin*, *T. jirovecii*, *T. mucoides*, and *T. pullulans*) were included in the analysis. The first finding of this study was that examination of the ITS2 region allowed for accurate identification of the yeast species, while the D1/D2 sequences were too conserved and could not discriminate between *T. dermatis* and *T. mucoides*. In addition, the authors reported that 4 out of 6 *T. dermatis* clinical isolates were misclassified by biochemical methods as *T. cutaneum*.

The limited ability of biochemical tests to provide reliable identification of clinical isolates of *Trichosporon* spp. was recently documented by Rodriguez-Tudela et al. (151). They compared the identification of 49 isolates of *Trichosporon* spp. using the amplification and sequencing of the ITS and intergenic spacer (IGS) regions with that of 4 different biochemical tests, including fermentation of different carbon sources, growth on nitrogen sources, growth at various temperatures, and the ability to hydrolyze urea. Not surprisingly, 26 isolates (53%) were misclassified at the species level according to sequencing analysis. Out of 15 *T. asahii* isolates, only 8 were correctly identified by biochemical tests, while 6 were identified as *T. mucoides* and 1 as *T. ovoides*. All isolates belonging to the *T. jirovecii*, *T. japonicum*, *T. montevidense*, and *T. domesticum* species were not able to be identified by classical

identification methodology. However, 6 *T. inkin* isolates out of 7 were correctly identified by biochemical tests.

Commercial systems based on phenotypic tests to provide identification of *Trichosporon* spp. are very limited in their accuracy because of either the limited number of species included in their databases or the inconsistent ability of their biochemical tests to accurately identify all 50 potential species within the genera. Considering the limitations of the phenotypic methods used to identify *Trichosporon* spp. at the species level, it is easy to understand why most reports refer only to the genus *Trichosporon* without determining the species or simply identify clinical isolates as *T. asahii* or non-*T. asahii*.

The lack of accurate laboratory tools for the complete identification of *Trichosporon* strains in clinical laboratories impairs the understanding of unique epidemiological and clinical features of these strains. This technical deficiency also impairs the ability to understand differences in clinical responses to conventional antifungal therapy, possibly related to the medically important species of this genus, previously named *T. beigeli* (10, 141, 185, 186).

#### Molecular Targets and Identification of *Trichosporon* Species in Cultures

**PCR-based methods.** DNA-based methods have been extensively used for the accurate identification of *Trichosporon* spp. (142). Indeed, the evaluation of specific nucleotide sequences can be a precise method to resolve taxonomic problems generated by the inconsistent phenotypic identification of *Trichosporon* species. In this regard, ribosomal genes represent particularly consistent evaluative markers and include alternating conserved regions (D1/D2 region of the 28S rDNA) and variable regions (ITS and IGS1 regions) that may be useful for species identification and phylogenetic studies (Fig. 1) (171, 175). Apparently, a combination of at least 2 of those markers should be used for the proper molecular identification of *Trichosporon* strains and analysis of their phylogenetic relationships.

Sugita et al. (175) constructed a phylogenetic tree using the small-subunit (SSU) region sequences of rDNAs from different pathogenic yeasts obtained from DNA libraries. The primer pair TRF and TRR, which amplifies part of the SSU region, was designed for the specific identification of the genus *Trichosporon* because these oligonucleotides do not amplify conserved regions in the ribosomal genes of other medically important yeasts besides *Trichosporon*.

Subsequently, Sugita et al. (174) have sequenced and analyzed the interspacer region (ITS1 and ITS2) genes of the rDNA from *Trichosporon* spp. and proposed 17 species and five varieties for this genus. Therefore, these authors concluded that the six medically relevant species could be accurately identified by their ITS sequences.

However, Sugita et al. (172) also analyzed the sequences of IGS1, which is localized between the 26S and 5S rDNA genes, in 25 isolates of *Trichosporon*. The IGS1 regions ranged in size from 195 bp to 704 bp. Comparative analysis of the nucleotide sequences suggested higher variation in the IGS1 region than in the ITS region. Therefore, the use of ITS region sequencing was considered unsuitable for the identification of the large number of recently described species of *Trichosporon*. In ad-

dition, these authors were also able to identify 5 different genotypes of *T. asahii* among 43 strains (172). Following this work, several others have reported the use of the IGS region to accurately identify *Trichosporon* spp. in cultures from several biological samples, such as skin, nails, urine, sputum, biopsy samples, and blood (25, 89, 104, 151, 169).

Based on the ITS1 and ITS2 sequences, Leaw et al. (104) developed an oligonucleotide array to identify 77 species of clinically relevant yeasts belonging to 16 genera. Probes for each yeast species were designed and included probes to identify *T. aquatile*, *T. asahii*, *T. cutaneum*, *T. inkin*, and *T. pullulans*, as well as a pan-probe for the identification of *T. asahii*, *T. aquatile*, and *T. inkin*. Those authors screened a collection of 452 yeast strains and verified that the array had 100% sensitivity and 97% specificity. There were 17 *Trichosporon* sp. type strains and 4 *T. cutaneum* clinical isolates included in the collection to validate the array and 4 other species, (*T. dermatitis*, *T. jirovecii*, *T. mucoides*, and *T. ovoides*) to test the assay specificity. The authors concluded that all *Trichosporon* spp. tested were correctly identified except for *T. ovoides*, which cross-hybridized with the pan-probe (104).

More recently, Makino et al. (112) used a real-time quantitative PCR (qPCR) assay to identify and quantify 9 yeast species, including *T. asahii* and *T. jirovecii*, in dairy product samples. They amplified conserved sequences of 28S rDNA D1/D2 domains with 6 primer sets and were able to identify all isolates that artificially contaminated fermented milk within a 5-h assay. These tools and DNA markers were useful for yeast identification and could possibly be used as a first screening tool for *Trichosporon* species in biological samples.

Other molecular markers have been tested for use in the identification of *Trichosporon* species. Biswas et al. (20) analyzed the sequences of the mitochondrial cytochrome b (cyt b) genes from 23 different fungal strains representing 42 species. They amplified a 396-bp fragment and observed that there were 141 variable nucleotide sites (35.6%) among *Trichosporon* strains. Twenty-two strains (from 11 different species) contained introns in their sequences. Analysis of the deduced protein sequences of the 396-bp fragments showed 34 variable amino acid sites (25.75%). *T. domesticum*, *T. montevidense*, *T. asahii*, *T. asteroides*, *T. gracile*, and *T. guehoae* all had identical amino acid sequences. Phylogenetic analysis revealed that all species of *Trichosporon* except *T. montevidense* and *T. domesticum* included species-specific cyt b genes. Using this approach, two isolates were then identified: *Trichosporon* sp. strain CBS 5581 was identified as *T. pullulans*, and the clinical isolate IFM 48794 was identified as *T. faecale* (20).

**Proteomics as a tool for fungal identification.** During the past few years, protein fingerprints have been used as genus and species signatures to discriminate among isolates and identify pathogens (58). Protein fingerprint analysis by mass spectrometry (MS), in which the protein content of a certain sample within a complex mixture is analyzed, is a widely used technique in proteomics studies. Mass spectrum profiles are acquired by mixing the samples with a matrix in which peptides are ionized using a laser. Thereafter, peptides are expelled from the metal target by high voltage and travel in a vacuum tube to be finally analyzed according to their mass/charge ratio ( $m/z$ ). This technique typically generates spectra of the most abundant proteins, ranging from

1,000 to 20,000 Da. The process to identify microorganisms in culture, including automated identification, is based on the matching of peaks from fingerprints deposited in a database, e.g., the matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS BioTyper system (Bruker Daltonics), with the peaks of the spectrum generated from the clinical sample. The more similar two spectra are, the more reliable is the match, and therefore, genus or species identification is obtained (21).

The MALDI BioTyper 2.0 software possesses more than 3,700 mass spectrum profiles in its database. Of those profiles, 274 are from fungi and 11 are related to *Trichosporon* spp. Of these 11, 6 are *T. asahii*, *T. cutaneum*, *T. debeurmannianum*, *T. inkin*, *T. mucoides*, and *T. ovoides* and 2 are *Trichosporon* sp. Another feature of this method is the time required for sample processing. One isolate can be processed and identified in less than 2 min, making this tool the next generation in microorganism identification (58).

Marklein and collaborators (115) have identified 285 isolates of medically important yeasts, including 3 isolates of *T. cutaneum*, using MALDI-TOF MS. Overall, the system identified 247 of the clinical isolates (92.5%); the remaining 20 isolates were identified only after complementation of the database with protein spectra representative of all type strains. All 3 isolates of *T. cutaneum* were properly identified by the API ID 32C system but not by the MALDI-TOF MS due to the absence of a reference protein spectrum characteristic for this species. After generation of a reference type spectrum for *T. cutaneum*, all 3 isolates were appropriately identified.

Recently, Bader et al. (13) tested the accuracy of MALDI-TOF MS using 2 different commercially available systems, MALDI BioTyper2 (Bruker Daltonics) and Saramis (AnagnosTec), for the identification of 1,192 clinical yeast isolates previously identified by morphological characterization and biochemical tests (API 20 C AUX and ID 32 C galleries). The congruence among all three identification methods was 95.1%, and all 4 isolates of *T. asahii* were properly identified by these three procedures.

Stevenson et al. (167) constructed a yeast mass spectrum library using the MALDI BioTyper2 (Bruker Daltonics) software. One hundred twenty-four type strains were initially evaluated for construction of the protein spectrum database library, but 15 were removed due to unacceptable spectra or discordant identification results after DNA sequencing, which occurred with 1 *T. asahii* strain and 3 *T. ovoides* strains. In order to validate the accuracy of the database library, 194 clinical isolates (23 species covering 6 genera) were tested, and 192 (99.0%) of the clinical isolates were properly identified by MALDI-TOF MS (ID score of <1.8), including 4 isolates of *T. asahii*, 1 of *T. coremiiforme*, and 3 of *T. mucoides*. MALDI-TOF MS should be considered a reliable technique to rapidly identify fungal species and may be used as an alternative to PCR-based methods, with the additional advantage of the lack of complicated DNA extraction (167).

It is therefore evident that the use of molecular methods to evaluate specific DNA sequences or proteins fingerprints is now considered mandatory to accurately identify strains belonging to the genus *Trichosporon*. Small clinical laboratories unable to run those tests should send their strains to reference

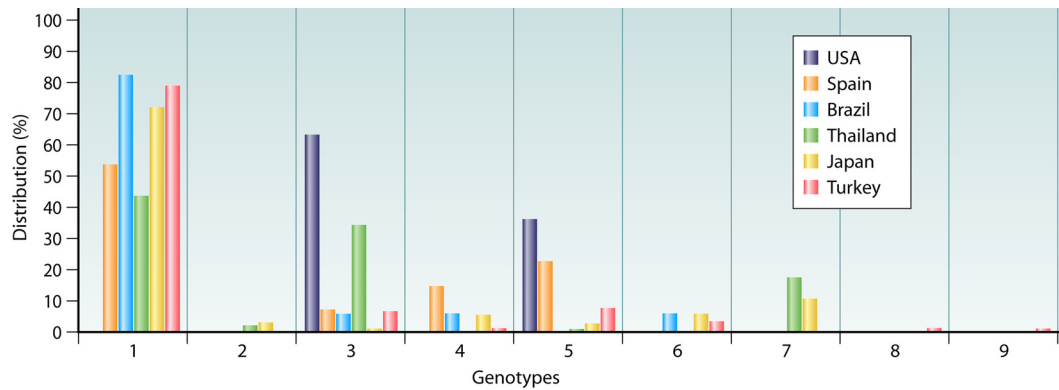


FIG. 2. Distribution of *T. asahii* genotypes, based on IGS1 sequences of clinical isolates, in the United States, Spain, Brazil, Thailand, Japan, and Turkey (25, 89, 119, 152, 169, 172).

laboratories to have the *Trichosporon* strains properly identified at the species level, particularly when such strains are obtained from deep-seated infections.

**Genotyping of *Trichosporon* spp., with an emphasis on the geographic distribution of *T. asahii* genotypes.** *T. asahii* is the most commonly isolated species from patients with invasive trichosporonosis and presents up to 9 different genotypes described around the globe, using the IGS1 sequence as the molecular marker (24, 119).

Sugita et al. were the first to describe 5 *T. asahii* genotypes, based on the diversity of the IGS1 sequences exhibited by *T. asahii* strains obtained from Japan, the United States, and Brazil (172). They found that among isolates obtained in Japan, 26 (87%) were genotype 1 or 3, while the others belonged to genotypes 2 and 4. The 13 *T. asahii* strains originally from the United States were representative of either genotype 3 or genotype 5, and the single Brazilian isolate was identified as genotype 3. No genotype 1 strains were found among the 13 North American strains tested.

Unlike the observations made with patients with superficial and invasive trichosporonosis, Sugita et al. reported that most *T. asahii* strains isolated from the homes of patients suffering with summer-type hypersensitivity pneumonitis were genotype 3, instead of genotype 1, which is predominant in patients. In the same study, those authors described 2 new genotypes of *T. asahii*, genotypes 6 and 7, both documented in patients with fungemia (169).

Rodríguez-Tudela et al. (152) also evaluated sequence polymorphisms of the IGS1 regions of *T. asahii* strains isolated from patients in Argentina, Brazil, and Spain. The authors were able to recognize 6 different genotypes within the collection of 18 *T. asahii* strains. The majority of the strains were representative of genotypes 1 and 5. Spanish strains exhibited all other genotypes, with the exception of genotype 2, whereas the 5 South American isolates belonged to genotypes 1 and 6 (152).

Chagas-Neto and collaborators analyzed the genotype distribution of 15 *T. asahii* isolates obtained from blood samples from patients in Brazil and found that the majority of these strains belonged to genotype 1 (86.7%). Only one isolate each belonged to genotypes 3 and 4, representing the first description of genotype 4 reported in a patient in South America (25).

Recently, two papers described the geographic distribution pattern of *T. asahii* genotypes in Turkey and Thailand based on

IGS1 sequences (Fig. 2). Kalkanci et al. reported that among 87 *T. asahii* clinical isolates from Turkey, genotype 1 was represented by 69 strains (79.3%), followed by genotypes 5 (7 strains, 8.0%), 3 (6 strains, 6.9%), 6 (3 strains, 3.4%), and 4 (1 strain, 1.1%) (89). By examining the genotyping of *T. asahii* strains performed worldwide, those authors noted that genotype 7 has been found only in Japan and that genotype 8 and the novel genotype 9 were described only in Turkey. Mekha et al. evaluated 101 *T. asahii* strains in Thailand and found that genotype 1 (45 strains, 44.5%) was predominant, followed by genotypes 3 (35 strains, 34.7%) and 6 (18 strains, 17.8%) (119).

Figure 2 summarizes the worldwide distribution of *T. asahii* strains obtained only from patients with superficial and invasive *Trichosporon* infections (25, 89, 119, 152, 169, 172). It is important to note that, with the exception of Turkey, Thailand, and Japan, a limited number (usually <30) of *T. asahii* strains have been genotyped in most studies published in the English language literature. Genotype 1 is the most abundant type of *T. asahii* strain in all regions, ranging from approximately 45 to 80%, except for the United States where it has never been identified. Genotypes 3 and 5 predominate in U.S. medical centers. Further studies are needed to clarify whether different genotypes of *T. asahii* isolates may present different features in terms of virulence, tissue tropism, or antifungal susceptibility.

#### ANTIFUNGAL SUSCEPTIBILITY TESTING FOR *TRICHOSPORON* SPP. AND PRINCIPLES OF THERAPY

Despite the increasing occurrence of invasive *Trichosporon* infections refractory to conventional antifungal drugs, there are few studies investigating the *in vitro* susceptibilities of clinical strains of *Trichosporon* spp. to antifungal compounds. Difficulties in species identification within the genus and the lack of standardized sensitivity tests *in vitro* contribute to the limited information available on this topic (24, 140).

##### Antifungal Susceptibility Tests

The Clinical and Laboratory Standards Institute (CLSI) document describing antifungal susceptibility testing of yeasts does not specifically address the genus *Trichosporon*. However, most available studies evaluating the susceptibility of *Trichosporon*

spp. to antifungal drugs *in vitro* use the CLSI broth microdilution method currently (2008) standardized for *Candida* spp. and *C. neoformans* (10, 132) or an adaptation of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) broth microdilution method, a recommendation originally proposed for the genus *Candida* (36).

Despite the fact that the CLSI methodology may be successfully adapted to test *Trichosporon* strains, several authors have suggested that the broth microdilution method is not satisfactory for detecting isolates resistant to amphotericin B, as it generates a narrow MIC variation within different clinical strains. Therefore, MIC breakpoints have not yet been established for amphotericin B assays (41, 49).

The majority of the studies examining sensitivity tests of *Trichosporon* spp. use the outdated nomenclature *T. beigeli* (28, 75, 141, 185). Therefore, sensitivities to antifungal drugs within different species of the genus *Trichosporon* are largely still not investigated. After the resolution of the genus *Trichosporon*, some authors have suggested that *T. asahii* is more resistant to amphotericin B and more sensitive to triazoles than other *Trichosporon* species (80, 89, 190).

Rodriguez-Tudela et al. (151) accurately identified 49 *Trichosporon* clinical isolates using IGS region sequencing and tested their susceptibility to antifungal drugs. Those authors demonstrated that all *T. asahii* isolates tested had amphotericin B MICs of  $\geq 2$   $\mu\text{g/ml}$ . They also observed that the majority of *T. coremiiforme* and *T. faecale* strains were also resistant to amphotericin B, whereas the other species tested had MICs of  $< 1$   $\mu\text{g/ml}$ . In a recent study coordinated by the same group, voriconazole and posaconazole were shown to be the 2 new antifungal drugs with the best *in vitro* antifungal activity against most *Trichosporon* spp. (8).

Chagas-Neto et al. (25) evaluated the *in vitro* activities for 22 bloodstream *Trichosporon* strains using the CLSI broth microdilution test. The authors found that the geometric means of MICs generated by triazoles against *Trichosporon* spp. were generally lower than those values obtained with amphotericin B. Voriconazole generated the lowest MIC values against all clinical strains (25).

Ruan et al. (154) evaluated the *in vitro* activities of 9 different antifungal drugs against 43 clinical isolates of *Trichosporon* species using broth microdilution. Voriconazole exhibited excellent *in vitro* activity against most of the strains tested, including isolates resistant to fluconazole. All isolates appeared to be resistant *in vitro* to all echinocandins. The authors concluded that most strains exhibited relatively high MIC values against amphotericin B.

### Antifungal Therapy

Despite the increasing relevance of the genus *Trichosporon* in contemporary medicine, the treatment of patients with trichosporonosis remains a challenge, as there are few data available on the *in vitro* and *in vivo* activities of antifungal drugs against clinically relevant species of the genus.

Superficial infections occasionally respond to topical antifungal agents, but white piedra may reoccur after suspension of the antimycotic. Consequently, some studies suggest that treatment of this disease should consist of hair removal followed by topical and/or oral antifungal therapy (96).

There are increasing data suggesting that amphotericin B has limited *in vitro* and *in vivo* activity against *Trichosporon* spp., including *T. asahii* strains (183, 193). Amphotericin B MIC values against *Trichosporon* spp., particularly *T. asahii*, are generally incompatible with serum levels normally achievable in patients receiving a conventional formulation of this polyene. Although some isolates can be inhibited by low concentrations of amphotericin B, fungicidal activity has not been observed in animal models or in neutropenic patients (3, 24, 25, 143, 179, 186, 189, 190).

In a study of 25 neutropenic patients who developed systemic trichosporonosis and were treated with amphotericin B, only four survived (81). Girmenia et al. reported the clinical outcomes for 55 patients with hematological diseases and disseminated trichosporonosis who were treated with amphotericin B. A clinical response to amphotericin B was documented in only 13/55 (24%) of the patients evaluated (62).

Triazoles seem to have better *in vitro* and *in vivo* antifungal activities against *Trichosporon* spp. than amphotericin B. Fluconazole was used as the initial therapy in 85% of 19 patients with invasive trichosporonosis documented in a single medical center in Taiwan, and the mortality rate was 42% (154). Suzuki et al. recently evaluated the clinical and therapeutic aspects of 33 cases of *Trichosporon* fungemia documented in patients with hematological malignancies. The authors clearly demonstrated that survival was longer for patients treated with an azole than for those treated with other drugs (179).

Voriconazole also has excellent *in vitro* activity against *Trichosporon* strains and may be useful for treating patients with trichosporonosis, including cases of acute leukemia and myelodysplastic syndrome with disseminated infection (12, 57, 106, 118, 140, 160).

Regarding the therapeutic role of 5-fluorocytosine (5FC) in trichosporonosis, the available data are limited and controversial. *In vitro* data suggest that a large proportion of *Trichosporon* strains may be less susceptible or resistant to 5FC (25, 119). In contrast, some authors report good results in the treatment of trichosporonosis with combination therapy with 5FC and amphotericin B (62). Consequently, at this time, we do not have substantial clinical data to safely recommend the utilization of 5FC to treat invasive trichosporonosis.

Finally, echinocandins alone have little to no activity against *Trichosporon* spp. and are not recommended for trichosporonosis treatment (188). Breakthrough *Trichosporon* infections have been reported in patients treated with echinocandins (caspofungin and micafungin) (35, 47, 188). Interestingly, a combination of echinocandin with amphotericin B or azoles appears to have some *in vitro* and *in vivo* synergistic antifungal effects (14, 161).

### SUMMARY AND CONCLUSION

*Trichosporon* spp. are basidiomycetous yeast-like anamorphic organisms, found in tropical and temperate areas of the globe and are widely distributed in nature. Although most *Trichosporon* strains isolated in clinical laboratories are related to episodes of colonization or superficial infections, this fungus has been recognized as an opportunistic agent causing emergent, invasive infections in tertiary care hospitals worldwide.

Invasive *Trichosporon* spp. have been documented mostly in cancer patients and in critically ill patients exposed to multiple invasive medical procedures. It is possible that the ability of *Trichosporon* strains to adhere to and form biofilms on implanted devices may account for the progress of invasive trichosporonosis, since this ability promotes escape from drugs and host immune responses. In addition, the presence of glucuronoxylomannan (GXM) in the cell walls of *Trichosporon* spp. and their ability to produce proteases and lipases are all factors likely related to the virulence of this genus.

The outdated taxon *T. beigeli* was replaced by several separate species, and the taxonomy of the genus was progressively modified by powerful molecular tools able to discriminate between phylogenetically closely related species. Currently, 50 different species of *Trichosporon* have been described by different authors, and 16 can cause human disease. Globally, *T. asahii* is the most common species causing invasive trichosporonosis and presents 9 different genotypes, based on the IGS1 sequence, that exhibit substantial variability in their geographic distribution worldwide.

Phenotypic methods for *Trichosporon* species identification are based on the characterization of micromorphological aspects of colonies and biochemical profiling. Performing a slide microculture to search for arthroconidia and testing for urease production are useful tools for the screening of *Trichosporon* spp. However, morphological aspects and biochemical tests do not allow the complete identification of *Trichosporon* isolates at the species level. Consequently, molecular methods, including sequencing of the IGS region, are necessary to obtain accurate results for the identification of *Trichosporon* strains.

Diagnosis of invasive trichosporonosis may be a challenge, and several molecular methods have been developed in the last 2 decades, including PCR-based methods, Luminex xMAP technology (a novel flow cytometry technique with potential for the detection of medically important species of fungi), and proteomics.

Despite the increasing relevance of the genus *Trichosporon* in contemporary medicine, treating patients with trichosporonosis remains a challenge given that we have little data available on the *in vitro* and *in vivo* activities of antifungal drugs against clinically relevant species of the genus.

In conclusion, disseminated trichosporonosis has been increasingly reported worldwide and represents a challenge for both diagnosis and species identification. Prognosis is limited, and antifungal regimens containing triazoles appear to be the best therapeutic approach. In addition, removal of central venous lines and control of underlying conditions should be considered to optimize clinical outcomes.

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**Arnaldo Lopes Colombo** is a Professor of Medicine at the Division of Infectious Diseases of the Federal University of São Paulo-UNIFESP, Brazil. He is currently the Dean of Research of the Federal University of São Paulo and Head of the Special Mycology Laboratory at the same university, a reference laboratory in Brazil and Latin America for yeast identification, typing, and antifungal susceptibility testing. Dr. Colombo obtained his M.D. from UNIFESP in 1983 and continued his residency training in internal medicine and infectious diseases there. He completed his fellowship training in medical mycology at the University of Texas Health Science Center at San Antonio, TX. He received his Ph.D. degree from UNIFESP in 1994. He has organized several multicenter surveillance studies to characterize the epidemiology of *Candida* and has actively participated in several surveillance programs to evaluate emergent fungal pathogens and antifungal resistance.



**Ana Carolina Barbosa Padovan** is a biologist and received her Ph.D. from the Department of Microbiology, Immunology and Parasitology of the Federal University of São Paulo-UNIFESP, Brazil, on the evolution of the Ascomycota fungi and the study of *Candida albicans* adherence genes and biofilm formation. She received postdoctoral training at Professor Roberto Kolter's lab in the Department of Microbiology and Molecular Genetics at Harvard Medical School, Boston, MA, on the study of microbial interactions that inhibit the expression of *Candida albicans* virulence factors. Currently, she is a postdoctoral fellow at the Special Mycology Laboratory-UNIFESP, and her interests involve the development and application of molecular diagnostics of emergent fungal pathogens and the interactions between the normal human microbiota and fungi in order to prevent superficial and invasive fungal infections.



**Guilherme Maranhão Chaves** is currently a Lecturer on Basic and Clinical Microbiology at the Department of Clinical and Toxicological Analysis at the Federal University of Rio Grande do Norte, UFRN, Brazil. He obtained his Ph.D. in molecular microbiology from University of Aberdeen, under the supervision of Professor Frank C. Odds, on the subject of oxidative stress and virulence in *Candida albicans*. He next received postdoctoral training at the Special Mycology Laboratory-UNIFESP on the epidemiological and genetic characterization of *Trichosporon* and *Candida* clinical isolates. His research interests are in molecular identification and genotyping and the characterization of virulence factors of *Candida* and emergent yeast pathogens.

