TRICHOSPORON SPECIES ISOLATED FROM THE PERIGENITAL REGION, URINE AND CATHETERS OF A BRAZILIAN POPULATION

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ABSTRACT

The present study aimed to 1) determine the colonization rates of medically important *Trichosporon* species on normal perigenital skin and 2) determine the isolation rates of *Trichosporon* spp. isolated from the urine and catheters of Brazilian patients hospitalized in the Intensive Care Unit (ICU). The overall colonization rate of *Trichosporon* spp. was 11.15% (112 isolates). The most common species isolated from normal perigenital skin was *T. cutaneum* (29.46%), followed by *T. asteroides* (20.53%), *T. ovoides* (15.17%), *T. inkin* (10.71%), *T. mucoides* (8.92%), and *T. asahii* (6.25%). From urine and catheters, *T. asahii* was the species most commonly isolated (76.5%; n =23), followed by *T. inkin* (16.6%; n = 5) and *T. asteroides* (6.6%; n = 2). In addition, the highest isolation rate occurred in subjects in the 71- to 80-year-old age range (36.7%; n= 11), followed by 61 to 70 (26.7%; n = 8), 51 to 60 (13.3%; n = 4), 31 to 40 (13.33%; n = 4), and 41 to 50 (10%; n =3). We concluded that 6 medically important species of the genus *Trichosporon* colonize the perigenital region in a normal population. The identification of these species is possible by means of classical methods but often requires repeated analyses repetitions due to difficulties in the assimilation process. In contrast, only 3 species of *Trichosporon* were isolated from urine and catheters.

Key words: *Trichosporon* spp.*,* epidemiology, perigenital skin, superficial mycosis.

INTRODUCTION

Trichosporon is a genus of anamorphic basidiomycetous yeast widely distributed in nature and which can form part of the human mycobiota (17). This fungus belongs to a medically important genus that includes the causative agents of deep-seated, mucosa-associated and superficial infections (13, 21, 34). These arthroconidial yeasts are well known as agents of white piedra (11, 13, 21, 34), but they are also reported to be opportunistic pathogens causing deep-seated and widely disseminated infections in immunocompromised patients (4, 9, 34).

Diagnosis of trichosporonosis is difficult and is often not confirmed until autopsy. A definitive diagnosis of disseminated trichosporonosis is usually established by histological examination of tissue samples obtained by biopsy as well as by detecting the causative pathogenic fungi in clinical samples (20).

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Identification of species from the *Trichosporon* genus by conventional methods is often difficult and is frequently inconclusive. This situation is further complicated by the lack of *in vitro* standardized sensitivity tests. These obstacles have resulted in the limited availability of information on the epidemiology, diagnosis and therapeutics of trichosporonosis (4, 11, 27). Gueho et al. (11) revised the taxonomy of the genus *Trichosporon*, whereas Sugita et al. (29, 30) proposed to classify the genus *Trichosporon* into 17 species. Seven morphological and biochemical patterns were recognized among clinical isolates associated with human infections: *T. asahii, T. asteroides, T. cutaneum, T. inkin, T. jirovecii, T. mucoides* and *T. ovoides*. Rodrigues-Tudela et al. (27) proposed a correct identification of the *Trichosporon* species by molecularly sequencing the intergenic spacer 1 (IGS1) of the rRNA gene. *T. asahii* appears to be more frequent in cases of systemic infection by *Trichosporon* species than in superficial infections (1, 2). In 2002, Sugita et al. (31) proposed classifying 25 species in the genus *Trichosporon* and suggested that 8 of these should be considered relevant as potential human pathogens, including two emergent species, *T. domesticum* and *T. montevideense*. Shortly thereafter, the same group published a study wherein they identified 36 *Trichosporon* species (32), including five new species proposed by Middelhoven et al. (18), *T. vadense, T. smithiae, T. dehoogii, T. scarabaeorum* and *T. gamsii*.

Traditionally, *Trichosporon spp*. are characterized by the presence of hyphae, pseudohyphae, blastoconida and arthroconidia, with morphological features that vary based on species and on their biochemical properties of carbon assimilation (1, 5, 10, 11, 13, 33, 34). There is a general consensus that molecular methods are required for accurate identification of this genus, but these methods are costly and not practical for most routine laboratories (26, 31). Despite the difficulties in identifying *Trichosporon* species by classical methodologies (morphological and biochemical characteristics), here, we use traditional techniques to analyze the epidemiological aspects of medically important *Trichosporon spp*. in a local Brazilian population.

MATERIAL AND METHODS

Patient selection and origins of *Trichosporon* isolates

From March 2006 to March 2008, 1004 normal male asymptomatic subjects were examined for superficial lesions in the Dermatology Department of São Paulo Hospital – Federal University of São Paulo (UNIFESP) and Heliópolis Hospital. The protocol was approved by the University Human Ethics Committee, and signed consent was obtained from each subject. These educationally diverse subjects, whose ages ranged from 03 to 70 years, included Caucasians, Blacks, Asians and others of mixed race. The square carpet technique used for the isolation of *Trichosporon spp.* was done based on the reports of Mariat and Tapia (14) and Mariat and Adan-Campos (15). At the moment of consultation, the subjects received a square of sterilized wool carpet to rub on their perigenital skin regions (scrotal, inguinal and perianal regions). The squares were then inoculated onto Sabouraud dextrose agar plates (Difco) supplemented with chloramphenicol (500 μg/ml). Culture plates were incubated at room temperature (25-28°C) and observed daily for growth for up to 4 weeks. All cultures suspected of being of positive for *Trichosporon* genus were suebcultured onto Sabouraud dextrose agar tubes. Gross morphology and microscopic characteristics were analyzed. Slide cultures of each isolate were also prepared for observation by optical microscopy (25).

During the same time period, 26 *Trichosporon* spp. isolated from urine and 4 *Trichosporon* spp. isolated from the catheters of 30 male patients hospitalized in the Intensive Care Unit (ICU), due to various health problems, were also studied. These patients were 30 to 80 years old and had presented serious physical and health conditions, but none had received a diagnosis of trichosporonosis. All patients were Caucasian with elementary school educational levels.

Biochemical and morphological identification of suspected *Trichosporon* isolates

All suspicious primary isolates were subcultured onto Sabouraud dextrose agar without antibiotics for 10 days at

room temperature. The cultures were first screened by colonial and microscopic morphology and then by analyzing their physiological characteristics of growth on carbon and nitrogen sources (assimilation patterns), growth at various temperatures, ability to hydrolyze urea, growth in 0.1% cycloheximide, and presence of appressorial cells (5).

RESULTS

Trichosporon species, which were identified by morphological and biochemical tests, were recovered from 112 (11.15%) out of the 1004 normal asymptomatic subjects examined. However, only 102 (91.1%) of isolates could be identified at the species level. Ten isolates (8.92%) were inconclusive to assimilation pattern tests.

The following species were identified: *T. cutaneum* (29.46%), *T. asteroids* (20.53%), *T. ovoides* (15.17%), *T. inkin* (10.71%), *T. mucoides* (8.92%), and *T. asahii* (6.25%). With respect to the identification of *Trichosporon* species by classical methodology, for some isolates it was necessary to repeat the assimilation test 2 to 3 times due to inconclusive initial results. In those cases, when a more intense inoculum was cultured onto the medium, a halo of assimilation could be better visualized.

The colonization rates of genus *Trichosporon* varied greatly by age and, to a lesser extent, by the race or educational level of the patient. Figures 1A and 1B show the percentages of isolates according to race and educational level.
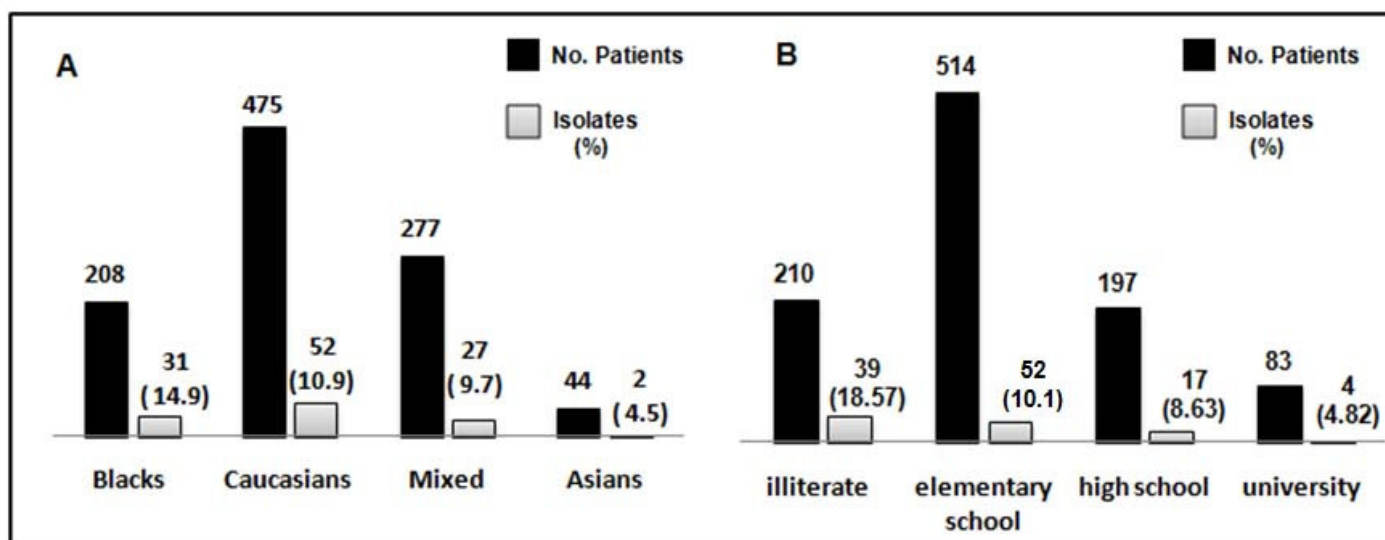

Figure 1. Number of individuals and colonization rates of Trichosporon spp. on perigenital skin according to race (A) and educational level (B). The percentage is relative to each group.

Table 1 shows the different *Trichosporon* species isolated from the perigenital skin area according to age. Skin colonization was higher among subjects aged 21 to 30 (48.2%) and 31 to 40 (25.0%) years, and all six relevant species of medical interest were present. Among the 112 *Trichosporon* spp. isolates, *T. cutaneum* was the most frequently isolated species (29.46%), followed by *T. asteroides* (20.53%), *T.* *ovoides* (15.17%), *T. inkin* (10.71%), *T. mucoides* (8.92%), and *T. asahii* (6.25%). Ten isolates (8.92%) could not be identified by phenotypic tests. *Trichosporon* isolates from the illiterate subject group comprised 18.57%, followed by subjects in elementary school (10.12%), high school (8.63%), and university graduates (4.82%).

Among the group of patients hospitalized in the ICU, 30

Trichosporon spp., 26 from urine and 4 from catheters, were identified. Each isolate was obtained from a different patient. *T. asahii* was the most isolated species (n=23; 76.66%), followed by *T. inkin* (n=5; 16.66%) and *T. asteroides* (n=2; 6.6%) (Table 2). Based on age ranges, the 71- to 80-year-old group represented 36.7% of the isolates, followed by subjected aged 61 to 70 (26.7%), 51 to 60 (13.33%), 31 to 40 (13.33%) and 41 to 50 (10%). All of these subjects had attended elementary school.

Table 1. Colonization rates of *Trichosporon* species isolated from the perigenital skin of male asymptomatic volunteers according to age.

SPECIES	AGES						TOTAL
	0 – 10 (n=59)	11- 20 (n=166)	21 – 30 (n=357)	31 – 40 (n=222)	41 – 50 (n=134)	51 – 70 (n=66)	N (%)
T. asahii		4	1	1		1	7 (6.25)
T. asteroides		4	11	6	1	1	23 (20.53)
T. ovoides		2	8	3	2	2	17 (15.17)
T. mucoides		4	1	2	3		10 (8.92)
T. inkin	2		7	1	1	1	12 (10.71)
T. cutaneum	1		20	11		1	33 (29.46)
Trichosporon spp.			6	4			10 (8.92)
TOTAL	3 (2.67%)	14 (12.5%)	54 (48.2%)	28 (25%)	7 (6.25%)	6 (5.35%)	112 (100)

Table 2. Isolation rates of *Trichosporon* species isolated from urine and catheters according to age.

SPECIES	AGE (years)					
	31 – 40	41 – 50	51 – 60	61 – 70	71-80	TOTAL
T. asahii	4	3	4*[2]	4	8	23 (76.66%)
T. inkin	-	-	-	3[1]	2[1]	5 (16.66%)
T. asteroides	-	-	-	1	1	2 (6.66%)
TOTAL	4(13.33%)	3 (10%)	4(13.33%)	8 (26.7%)	11 (36.7%)	30 (100%)

*(n) = isolated from catheter (2 *T. asahii* and 2 *T. inkin*)

DISCUSSION

This study identifies both biochemical and morphological aspects of the largest number of *Trichosporon* samples by species level (121 from superficial sites and 30 deep seated).

Although *Trichosporon* species include a heterogeneous group of arthroconidia-forming yeasts, this characteristic by itself has very low distinctive value in the identification of different species, since other yeasts are also able to form these structures (4, 12). Conventional carbon and nitrogen

assimilation tests (auxonograms) are easy to perform but frequently need to be repeated several times until the results are confirmed. If classical tests are not sufficient to identify *Trichosporon* species, molecular approaches must be used (20, 23, and 27).

This is the first time since the creation of the new classification scheme of the genus *Trichosporon* (27, 28, 32) that 6 of the 7 medically important species have been isolated and identified from the normal perigenital region. Although the natural habitat of *Trichosporon* is considered to be the soil, little is known about its colonization in normal skin (6). In our previous study in 1980 on genital white piedra among 300 young students, we isolated 33% of *Trichosporon yeasts from normal perigenital skin and 25% from scrotal hairs using the carpet* square technique. On that occasion, the fungus isolated was identified as *T. beigelii (7). In* Brazil, *T. inkin and T. asahii* have also been reported to colonize the hair shaft in the anal and perianal regions of HIV-positive patients (22). *T. cutaneum* was recently identified in an outbreak of scalp white piedra in a Brazilian child daycare center (28).

In the present study, we found that the genus *Trichosporon* colonizes the perigenital *region* of the normal population at a rate of 11.15%. In addition, we found that the most isolated species included 6 of the 7 medically important *Trichosporon* species. *T. cutaneum (29.46%)* and *T. asteroides (20.53%)* were the most isolated species, followed by *T. ovoides (15.17%), T. inkin (10.71%), T. mucoides (8.92%), and T. asahii (6.25%).* Young adult subjects aged 21 to 30 years presented the highest colonization rate (54 isolates, 48.21%), followed by the groups aged 31 to 40 (28 isolates; 25.0%), 11 to 20 (14 isolates; 12.5%), 51 to 70 (6 isolates; 5.35%), 41 to 50 (7 isolates; 6.25%), and 0 to 10 (3 isolates; 2.67%) years. All 6 species were isolated from the groups aged 21 to 30 and 31 to 40 years, indicating that, in young adult males, the colonization rates of *Trichosporon* spp. are higher and that almost all species of medical interest are present. The role of these species as microbiota in the perigenital region is unknown but constitutes a focus for possible contamination of catheters and urethrae.

Based on our results, we conclude that 6 out of the 7 medically important *Trichosporon* spp. species are part of the normal perigenital skin mycobiota in males of all ages. Although we cannot determine whether this site is the main source of infection by the use of catheters, it is a possible source of urinary tract infection due to contact of the penis (urethra) with this region.

Our study also showed that *T. asahii* was the species most frequently isolated from urine and catheters (76.6%), which is in contrast to what was found for the perigenital skin region (6.25%). The ICU patients studied herein presented several important factors that may contribute to the disruption of the skin-mucosa barrier; however, none of them had been diagnosed with trichosporonosis. Girmenia et al. (8) reported that the most common underlying conditions related to trichosporonosis were hematological diseases, peritoneal dialysis and solid tumors. These risk factors are often found in ICU patients. Moretti-Branchini et al. (19) reported two Brazilian cases of *Trichosporon*-invasive infections in patients undergoing bone marrow transplantation. One of these patients had an intravascular catheter tip culture test return positive for T. inkin; the second patient was neutropenic, and the culture was identified as T. asahii. Ribeiro et al. (24) performed sequencing analysis of the IGS1 region of *Trichosporon* spp. isolated from Brazilian patients. This analysis revealed that different *Trichosporon* spp. could be grouped into distinct clades and that, in superficial regions, *T. asahii* was the predominant species (43%), followed by *T. faecale (24%) and T. inkin (14%).* Furthermore, *T. asahii (83%) and T. inkin (17%)* were isolated from deep sites. Chagas-Neto et al. (3) analyzed bloodstream infections due to *Trichosporon* spp. by sequencing IGS1 and found that *T. asahii* was the prevailing species, followed by *T. asteroides, T. coremiiforme and T. dermatis.*

Invasive infections caused by Trichosporon spp. are commonly associated with the use of central venous catheters (CVC. In the present study, we identified four isolates from CVC, with *T. inkin and T. asahii* being identified twice in each. In urinary infections, *T. asahii* was the most isolated species.

Our data agree with previous reports demonstrating that *T. asahii* is the most frequently isolated species from patients presenting risk factors (2, 13, 16, 33-35).

In conclusion, this study has demonstrated that 6 out of 7 the medically important *Trichosporon* species are present in the perigenital region of normal male subjects and that the type of species present correlates with age. In urine and catheters of ICU patients, 3 *Trichosporon* species were isolated, but *T. asahii* was the most frequently isolated species.

ACKNOWLEDGEMENTS

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