

## *Trichosporon* isolation from human ungueal infections: is there a pathogenic role?\*

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**Abstract:** BACKGROUND: Although dermatophytes are considered the major cause of onychomycosis, many reports have incriminated non-dermatophyte moulds and yeasts in the disease's etiology. Successive *Trichosporon* isolation from onychomycosis has led to the genus being suspected as a nail primary pathogen.

OBJECTIVE: To determine the prevalence of *Trichosporon* isolation in onychomycosis patients who attended a mycology diagnostic service in Rio de Janeiro, Brazil, between January 2003 and December 2006. The study also includes a worldwide review on *Trichosporon* isolation prevalence in ungueal disease, emphasizing *T. ovoides*.

METHODS: This retrospective study was conducted with the support of staff from the Mycology Laboratory at the Dermatological Service of Rio de Janeiro's Santa Casa da Misericórdia (MLDS).

RESULTS: Mycological analysis provided positive results equaling 47/5036 (0.93%) for *Trichosporon* spp.; obtained mainly as a single agent (72.35%), and from mixed cultures (27.65%;  $\chi^2=6.397$ ;  $p=0.018$ ). The great majority belongs to the *T. ovoides* species (91.5%;  $n=43$ ), obtained as a single isolate (74.41%;  $n=32/43$ ;  $\chi^2=7.023$ ;  $p=0.014$ ).

CONCLUSIONS: Although *T. ovoides* is classically associated as an etiologic agent of white piedra, this study highlights its potential as a human nail disease pathogen. Our study opens doors for future epidemiologic and virulence factors aimed at determining whether *T. ovoides* is an important causative agent of onychomycosis in Brazil.

**Keywords:** Diagnosis; Nail diseases; *Trichosporon*; Trichosporonosis.

### INTRODUCTION

Onychomycosis is characterized by nail thickening and dystrophy, and may also affect the surrounding skin. Due to its prevalence, it represents a public health problem which affects mainly the elderly and, especially, diabetic subjects. In addition to esthetic and emotional harm, it can be painful and prevent daily activities and/or aggravate pre-existing conditions.<sup>1,2</sup> The frequency of yeast isolation from onychomycosis has increased over the last years, which has been attributed to both local and systemic factors including continuous finger exposure to humidity, antibacterial drug prophylaxis and treatment with immunosuppressive drugs.<sup>3</sup>

Although dermatophytes are considered the major cause of onychomycosis, many reports have incriminated non-dermatophyte moulds and yeasts in the etiology of ungueal disease.<sup>4,5,6</sup> Among yeasts, the *Candida* genus is frequently isolated from such lesions whereas moulds are represented by *Fusarium*, *Aspergillus*, *Alternaria*, *Curvularia* species and *Scytalidium dimidiatum*.<sup>2,6</sup>

The *Trichosporon* Behrend genus has been continuously revised by biochemical and molecular tools, resulting in the establishment of the 6 major human pathogens: *T. asahii*, *T. mucoides*, *T. ovoides*, *T. asteroides*, *T. cutaneum* and *T. inkin*<sup>7,8,9,10</sup>, plus 15 other species of medical significance.<sup>11</sup> These are causative agents

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of benign superficial lesions, classically the white piedra, determined by the presence of head and pubic hair nodules due to *T. ovoides* and *T. inkin*, respectively.<sup>7,8</sup> Furthermore, frequent reports of allergic pneumonia and invasive *trichosporonosis* have been published, as reviewed by Chagas-Neto et al.<sup>5</sup> and Colombo et al.<sup>11</sup> The considerable frequency of *Trichosporon* spp. isolation from onychomycosis has led to the genus being suspected as a nail primary pathogen, other than a secondary colonizer following dermatophyte nail infection.<sup>12,13</sup>

This study sought to determine *Trichosporon* isolation prevalence in onychomycosis patients who attended a mycology diagnostic service in Rio de Janeiro, Brazil. It also includes a worldwide review on *Trichosporon* spp. isolation prevalence in ungual disease, focusing on its potential pathogenic role.

## MATERIALS AND METHODS

This retrospective study was conducted by staff from the Mycology Laboratory at the Dermatological Service of Rio de Janeiro's *Santa Casa da Misericórdia* (MLDS) between January 2003 and December 2006. A total of 7,527 patients were advised to seek a mycology diagnostic service because they had hair, skin and/or ungual lesions, suggesting superficial mycosis, as evaluated by physicians from Rio de Janeiro. They all underwent further analysis for fungal agents by the MLDS staff. The study included 47 patients, all of whose mycological exams returned positive results for *Trichosporon*.

### Mycological Analysis

To obtain the specimens used in this study, MLDS biologists scraped fingernail and/or toenail lesions with a surgical blade, as classically described in dermatology guidelines. The material was immediately sent for examination by direct KOH treatment and inoculation in Sabouraud glucose agar, with and without chloramphenicol and cycloheximide (BD, New Jersey, US). All tubes were cultivated at 25°C for up to 30 days. Direct examination was considered positive for fungus whenever yeast-like and/or hyphae were seen under light microscopy. Yeasts suggestive of the *Trichosporon* genus were purified and preserved in Skim Milk (DIFCO, USA) solution at 20% (w/v) for further identification, based on their morphological and biochemical characteristics. To identify species, growth at 37°C, assimilation tests and cycloheximide tolerance tests were performed.<sup>14</sup> Identification was carried out via culture, first by screening colonial and microscopic morphology, then by analyzing the species': physiological growth characteristics on carbon and nitrogen sources (assimilation patterns), ability to hydrolyze urea, growth in 0.1% and 0.01% cyclo-

heximide, and the presence of appressorial cells, as previously detailed.<sup>15</sup> Reference microorganisms *T. ovoides* INCQS 40270 (ATCC 90040) and *T. inkin* INCQS 40266 (ATCC 18020), acquired from the American Type Culture Collection and maintained in the Culture Collection of *Instituto Nacional de Controle de Qualidade* (INCQS), were used as control to identify the species in this investigation's various tests.

### Ethical aspects

The research protocol was submitted to the Research Ethics Committee at the *Santa Casa de Misericórdia* of Rio de Janeiro and approved on June 16<sup>th</sup>, 2007 (protocol number 016/07).

### Statistical analysis

Statistical analysis was performed using the Epi-Info statistics program (version 6.0). The chi-square 2 test was applied to obtain independence between proportions. Using the binomial test, the relationship between the participants' clinical characteristics and of *Trichosporon* spp. isolation was assessed. The level of significance adopted for statistical inference was 5%.

## RESULTS

Laboratory analysis revealed an onychomycosis prevalence of 66.9% (5036/7527), as determined after direct examination and positive culture. Forty-seven patients had positive results for *Trichosporon* spp., all from a single infection site (toenail or fingernail); the majority were women (35/47;  $p = 0.001$ ), aged 50-70 years, living in the metropolitan region of Rio de Janeiro. Likewise, isolating this genus among all patients diagnosed with onychomycosis presented a prevalence of 0.93% (47/5036). *Trichosporon* isolation varied according to patients' sex and anatomic site; women's fingernails were affected more frequently (24/47) than both, men (12/47) and women's toenails (11/47; binomial test  $p = 0.008$ ). *T. ovoides* isolation yielded a heterogeneous distribution which varied according to age groups ( $X^2 = 31,396$ ;  $p = 0.0001$ ), entailing a major concentration in individuals aged 61-70 years and a significant reduction among two groups: the 11-20 and 81-90 age groups ( $p < 0.05$ ; Table 1).

*Trichosporon* was obtained mainly as a single agent (34/47; 72.35%), but also from mixed cultures (13/47; 27.65%; Table 1), with one additional yeast, mould or dermatophyte ( $X^2 = 6.397$ ;  $p = 0.018$ ). The vast majority of all *Trichosporon* recovered were *T. ovoides* (91.5%;  $n=43$ ) as a single isolate (74.41%;  $n= 32/43$ ;  $X^2 = 7.023$ ;  $p = 0.014$ ), while the remaining could not be identified to the species level. *T. ovoides* was also detected in mixed cultures from ungual scraping, along with dermatophytes (two *Tricophyton*

**TABLE 1:** *Trichosporon* spp. according to onychomycosis site (fingernails or toenails), number of isolates and culture results of patients from Rio de Janeiro; obtained between January 2003 and December 2006

Onychomycosis site	<i>Trichosporon ovoides</i>	<i>Trichosporon</i> spp.	Number (%) of Isolates
<b>Ungueal</b>	43	4	47
Fingernails	28 (87,5%)	4 (12,5%)	32 (68.1)
Toenails	15 (100%)	-	15 (31.9)
<b>Culture</b>			
Mixed	11	2*	13 (%)
Single	32	2	34 (%)
<b>TOTAL</b>	43	4	47 (100)

\*T. mentagrophytes and *Candida* spp.

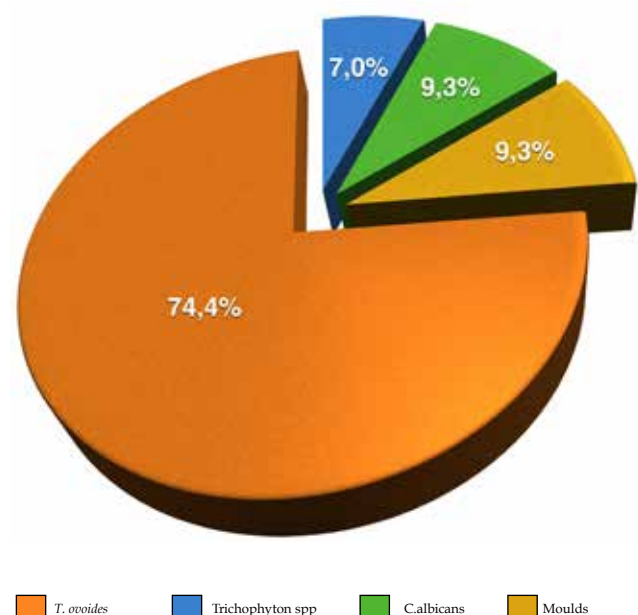
*rubrum* and one *Tricophyton mentagrophytes*; 3/43), yeast (four *Candida* spp.; 4/43) and moulds (two *Fusarium* spp. and two *Scytalidium dimidiatum*; 4/43). All fungi recovered from toenails resulted in the identification of *T. ovoides*. The non-identified species from the genus (n=4) were either isolated together with *Candida* spp. and *T. mentagrophytes* (one each), or as the only fungi recovered (n = 2).

## DISCUSSION

In 1976, in Colombia, Restrepo and Uribe were the first to suspect *Trichosporon* was a human nail pathogen. Later, in 1984, Fusaro and Miller described the first case of onychomycosis by this genus in a normal, healthy man living in the US.<sup>16,17</sup> Following these pioneering reports, other authors, studying different populations, uncovered data supporting the same hypothesis.<sup>12,13,18-25</sup> In addition, the first report of *T. montevidense* involvement in a Japanese monkey (*Macaca fuscata*) with onychomycosis revealed that the genus was potentially a nail pathogen in vertebrates other than humans.<sup>26</sup> However, establishing *Trichosporon* as a human nail pathogen is difficult because of its frequent isolation, either: as a member of the normal skin surface microbiota and appendages, or from mixed cultures along with classical nail pathogens such as *Tricophyton rubrum*.<sup>2,12,16</sup> Although *T. ovoides* is classically recognized as one of white piedra's etiologic agents, this investigation highlights its isolation as well as other *Trichosporon* species from human nail disease worldwide, discussing its potential pathogenic role.

Onychomycosis is the most common human nail disease with an overall frequency ranging from 18% to 73%, influenced by factors including geographical location, climate, cultural habits and genetic background.<sup>2,3</sup> Our study has a high prevalence (66.9%), probably due to Rio de Janeiro's hot and humid climate. While we detected a 0.93% frequency for *Trichosporon* isolation from onychomycosis, mainly *T. ovoides* single isolates (74.4%), other authors have

reported frequencies varying from 0.54% to 8% for the same genus, in different Brazilian populations (Graph 1 and Table 2). Interestingly, in Rio de Janeiro State, two reports revealed great variation: one found a similar frequency to ours (0.87%)<sup>20</sup>, whereas the other study's was higher (8%)<sup>2</sup> – close to rates mentioned in reports on different populations, for instance in Turkey (9.49%)<sup>23</sup>, Korea (10.1%)<sup>12</sup> and Nigeria (10.3%).<sup>18</sup> Apart from Brazil, in South America, only Colombians have been studied in two distinct decade reports, showing diverse frequencies of 5.53% and 1.08%.<sup>16,27</sup> Intermediate numbers have been obtained in different countries and in other Brazilian states, as described in table 2.



**GRAPH 1:** Frequency of *Trichosporon ovoides* obtained from onychomycosis as a single isolated yeast (74.4%) or along with other fungi (dermatophytes, *Candida albicans* or moulds)

**TABLE 2:** Trichosporon spp. and its species isolation frequency; description of other fungi recovery from human nail disease according to different authors and countries/localities; and their comments on the evidence of nail pathogenicity for this genus

Author/Year	Location/Country	Number of Study Patients or tNail samples	Trichosporon spp. Frequency (%) <sup>*</sup>	Trichosporon Species as Identified**	Other Yeast	Dermatophytes	Non-dermatophyte moulds	Is there evidence of Trichosporon spp. as a nail pathogen?
Restrepo & Uribe/1976	Colombia	524	5.53	T. cutaneum T. capitatum	Candida spp.	Yes	Geotrichum capitatum G. candidum	Yes
Fusaro & Miller 1984	United States	Case Report	n.a.	Trichosporon beigeli	-	-	-	Yes, "the first reported case of nail infection with this fungus in the United States".
Han et al. 2000	Korea	2591†	10.1	T. beigeli	- Malassezia pachydermatis - Candida spp. - Rhodotorula spp.	- Tricophyton rubrum - Tricophyton mentagrophytes	Aspergillus spp. Fusarium spp. Penicillium spp. Dematiaceae Other moulds	Yes, the second most commonly isolated fungus after T. rubrum.
Oyeka & Ugwu 2002	Nigeria	78	10.3	T. cutaneum	C. albicans	Microsporum gypseum T. mentagrophytes T. rubrum	Fusarium spp. Scopulariopsis breviscaulis	Yes
Pontes et al. 2002	João Pessoa/Brazil	306	3.6	Trichosporon spp.	-	-	-	Yes, special mention to DLSO**
Aratijo et al. 2003 <sup>a</sup>	Rio de Janeiro/Brazil	595	0.87	Trichosporon spp.	-	-	-	No
Aratijo et al. 2003 <sup>b</sup>	Rio de Janeiro/Brazil	400	8	Trichosporon spp.	-	-	-	Yes, except when isolated along with dermatophytes.
Sverigaard & Nilsson/2004	Denmark	948	2.75	T. cutaneum	-	Yes	Yes	Yes, except when isolated along with other fungi.
Alvarez et al. 2004	Cali/Colombia	183	1.08	T. beigeli	C. albicans C. parapsilosis C. guilliermondii C. famata	T. rubrum T. mentagrophytes	- Fusarium spp. - Scytalidium spp.	No
Gunduz et al. 2006	Manisa/Turkey	116	9.49	Trichosporon spp.	-	-	-	Yes, "the main pathogen"

\*Either single or mixed culture isolation.

\*\* Trichosporon species were described as published, therefore with limitations concerning accurate species determination.

† Nail samples

DLSO = lateral subungual onychomycosis

n.a. = not applied

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**TABLE 2: Trichosporon spp. and its species isolation frequency; description of other fungi recovery from human nail disease according to different authors and countries/localities; and their comments on the evidence of nail pathogenicity for this genus**

Author/Year	Location/ Country	Number of Study Patients or tNail samples	Trichosporon spp. Frequency (%) <sup>*</sup>	Trichosporon Species as Identified**	Other Yeast	Dermatophytes	Non-dermatophyte moulds	Is there evidence of Trichosporon spp. as a nail pathogen?
Mügge et al. 2006	Leipzig/ Alemanha	5077†	0.93	T. capitatum T. mucoides T. cutaneum Trichosporon spp.	C. guilliermondii C. tropicalis Candida spp.	T. rubrum T. interdigitale	-	Yes, as the fourth most common yeast.
Souza et al. 2007	Maringá/ Brazil	926	0.54	T. asahii T. inkin T. mucoides T. ovoides	C. albicans C. parapsilosis C. guilliermondii C. famata, C. krusei, C. lypholitica, C. lusitaniae, C. rugosa, C. stellatoidea, C. tropicalis. M.pachydermatis,	Dermatophytes	Other moulds	No
Souza et al. 2008	Goiania/ Brazil	1282	1.2	Trichosporon spp.	C. albicans Geotrichum	T. rubrum T. mentagrophytes Epidermophyton flocosum	Aspergillus spp. Fusarium spp.	No
Koksal et al. 2009	Istanbul/ Turkey	5722	1.2	Trichosporon spp.	C. albicans	T. rubrum T. mentagrophytes	-	Yes, "particularly in fingernails... In adults, males were more frequently infected with Trichosporon sp. than females".
Tajr-Aldeen et al. 2009	Qatar	7	n.a.	T. asahii T. dohaense	-	-	-	Yes
Present work 2015	Rio de Janeiro/ Brazil	5036	0.93	T. ovoides Trichosporon spp.	Candida albicans	- Tricopyrton rubrum - Tricopyrton menta- grophytes	- Fusarium spp. - Scytalidium dimid- iatum	Yes

\*Either single or mixed culture isolation.  
 \*\* Trichosporon species were described as published, therefore with limitations concerning accurate species determination.  
 † Nail samples  
 DLSO = lateral subungual onychomycosis  
 n.a. = not applied

Although previous investigations have suggested *Trichosporon* is a nail pathogen – or even refer to it as such – most do not identify this genus to the species level or only describe *T. beigelii* (Table 2). Our study identified *T. ovoides* as this genus' exclusive species, isolated from nail disease patients. *T. ovoides* was obtained mainly from women's fingernails, as previously reported, probably due to their frequent exposure to water and house cleaning products.<sup>24</sup>

Although *T. ovoides* is not incriminated as the *Trichosporon* genus systemic disease-associated pathogen, like *T. asahii*, we were able to isolate this species at a considerable frequency, as a single microorganism, from onychomycosis in patients living in Rio de Janeiro. It is difficult to discuss our data and the aforementioned publication in terms of *Trichosporon* species. Although this genus has been widely reviewed, diagnosing it accurately remains a challenge. Consequently, it has been broken down into two names: *T. beigelii* and *T. cutaneum*.<sup>5,7,8,11,12</sup> In fact, Guehó et al.<sup>7</sup> demonstrated that the term *T. ovoides* Behrend should replace *T. beigelii*. The latter species was discussed by some of the authors mentioned in table 2.<sup>5,12,17</sup> However, in line with the reclassification<sup>7</sup>, these isolates could actually be any of the genus' six pathogens, including *T. ovoides*. Only one previously published study has recovered *T. ovoides* as a single yeast from an onychomycosis patient's nail (in Maringá, Paraná, Brazil).<sup>28</sup>

## CONCLUSIONS

We were unable to perform serial nail collection from each patient to investigate *T. ovoides*' long-term colonization, as Han et al. did<sup>12</sup> and as strongly recommended by Meireles et al. for diagnosing onychomycosis.<sup>29</sup> Nor did we conduct molecular sequencing for species identification, and these are limitations we acknowledge.<sup>11</sup> Nevertheless, unlike other reports, we were able to incriminate *T. ovoides* as the single agent of nail infection. Hence, we propose that this species can play a role in human nail disease. Our study invites future epidemiologic and virulence approaches to determine whether *T. ovoides* is an important causative agent of onychomycosis in Brazil. □

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