



Identification of *Malassezia* yeast species isolated from patients with pityriasis versicolor^{*}

Identificação de espécies de malassésia na pitiríase versicolor em um serviço de dermatologia do sul do Brasil

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Abstract: Pityriasis versicolor (PV) is a disease with worldwide distribution. Twelve different species of *Malassezia* yeast have been described. The objective of this study was to determine which species of *Malassezia* are more prevalent in patients with pityriasis versicolor. Samples were collected by scraping the lesions of 87 patients with a clinical suspicion of pityriasis versicolor. The samples were then submitted to fungal microscopy and culture to identify the species. The species found were: *Malassezia sympodialis* (30%), *Malassezia furfur* (25.7%), *Malassezia globosa* (22.7%), *Malassezia restricta* (12.1%), *Malassezia obtusa* (7.6%) and *Malassezia slooffiae* (1.5%).

Keywords: Dermatology; Dermatomycoses; Fungi; Mycoses.

Resumo: A pitiríase versicolor é uma doença de distribuição universal. Existe a descrição de 12 espécies de malassezia. O objetivo deste estudo foi determinar quais as espécies de malassezia mais prevalentes nos pacientes com pitiríase versicolor. Foram realizadas as coletas através de raspado das lesões nos pacientes com suspeita clínica de pitiríase versicolor e posterior exame micológico e cultural para identificação final da espécie. Foram coletadas amostras de 87 pacientes. Quanto às culturas, 30% foram de *Malassezia sympodialis*, 25,7% de *Malassezia furfur*, 22,7% de *Malassezia globosa*, 12,1% de *Malassezia retrita*, 7,6% de *Malassezia obtusa* e 1,5% de *Malassezia slooffiae*.

Palavras-chave: Dermatologia; Dermatomicoses; Fungos; Micoses

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Pityriasis versicolor (PV) is a disease with worldwide distribution.^{1,3} The diagnosis of PV is based on clinical findings and confirmed by direct microscopy.² Culture and molecular analysis of *Malassezia* microflora may be used to identify different *Malassezia* species.⁴ In 1996, Guillot et al.

described a method for identifying species of *Malassezia* based on culture and biochemical reactions.⁵ Currently, twelve different species of *Malassezia* have been described; however, not all are clinically relevant in humans.⁶ The objective of this study was to determine which species of *Malassezia* were most prevalent in the population of patients with pityriasis versicolor receiving care at the dermatology outpatient clinic of the Federal University of Health Sciences of Porto Alegre (UFCSPA).

A cross-sectional study was conducted in which patients with PV being seen at the dermatology outpatient clinic over a one-year period were invited to participate. Patients under 16 years of age, those who had used systemic or topical antifungal medication in the preceding month and patients known to have an immunosuppressive disease were excluded from the study. The study was approved by the Internal Review Board of UFCSPA under approval letter #383/07. Data analysis was performed using the SigmaStat software. Student's t-test and the chi-square test were used for continuous and categorical variables, respectively. The Mann-Whitney/Wilcoxon Two-Sample Test (Kruskal-Wallis test for two groups) was used to compare quantitative variables between two independent groups. The diagnosis of pityriasis versicolor was confirmed by clinical observation and direct microscopy. For the primary isolation of the fungi in the material collected, Sabouraud agar and Dixon's medium were

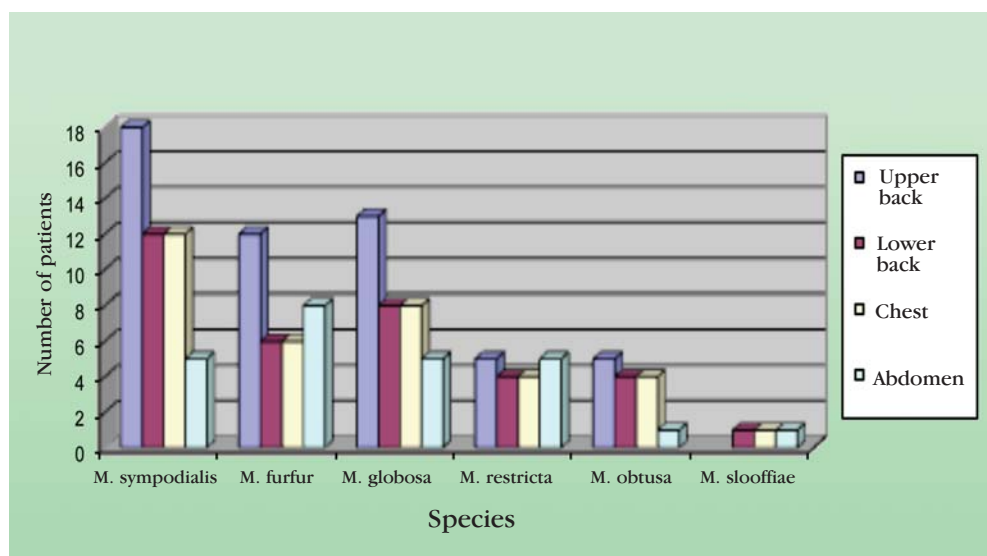
used, with the addition of olive oil in both cases. After seeding, the material was incubated at 32-35°C for 3-

6 days. Glabrous colonies of a creamy-yellow color and a furrowed surface were observed, the reverse of the colony also being creamy-yellow in color. Following primary isolation, final identification of the species was made using the methodology outlined by Guillot et al. (1996), which is based on the phenotypic characteristics of the species: the capacity to grow in the absence of lipids, production of the enzyme catalase, description of the micromorphology and the ability to assimilate different concentrations of Tween (20, 40, 60 and 80) in Sabouraud agar containing 0.05% of chloramphenicol and 0.05% of cycloheximide.⁵ The Tween assimilation test was performed

using a suspension of colonies inoculated into a plate containing Sabouraud agar supplemented with 0.05% chloramphenicol and 0.05% cycloheximide. Each polysorbate (Tween 20, 40, 60 and 80) was added to fill up equidistant wells made in the inoculated agar. The plates were then incubated at 32°C for 5-7 days.

After this period, the growth around each well, indicating assimilation of the substrate and a positive result, was observed. The set of positive results permitted differentiation between the species.

Samples were collected from 87 patients, 51 (58.6%) female and 36 (41.4%) male. The mean age of the patients was 31 ± 15 years. With respect to the site of the lesions, 90% of the patients had lesions on more than one part of the body, the most common being the lower back (53 patients) and the upper back (36 patients). Sixty-seven patients (77%) in whom the condition was clinically suspected tested positive at



GRAPH 1: Distribution of the site of pityriasis versicolor lesions according to the isolated species of *Malassezia* yeast ($p = 0.379$)

TABLE 1: Duration and recurrence of the lesions according to the species of *Malassezia* yeast isolated

	<i>M sympodialis</i>	<i>M furfur</i>	<i>M globosa</i>	<i>M restricta</i>	<i>M obtusa</i>	<i>M slooffiae</i>
Duration of the lesions in months						
p = 0.716						
1-3	15.8%	6.2%	22.2%	25%	0	0
3-6	15.8%	18.7%	33.3%	25%	0	100%
6-9	5.2%	18.7%	0	0	0	0
9-12	15.8%	12.5%	11.1%	12.5%	20%	0
More than 12	47.3%	43.8%	33.3%	37.5%	80%	0
Recurrences						
p = 0.300						
None	10	6	10	3	2	1
1-5 times	7	9	4	4	0	0
5-10 times	1	1	1	1	1	0
More than 10 times	2	1	0	0	2	0

direct mycological examination, while 66 (75%) had a positive mycological culture. With respect to the cultures, 30% consisted of *Malassezia sympodialis*, 25.7% *Malassezia furfur*, 22.7% *Malassezia globosa*, 12.1% *Malassezia restricta*, 7.6% *Malassezia obtusa* and 1.5% *Malassezia slooffiae*. Figure 1 shows the site of lesions in patients in relation to the species of *Malassezia* isolated in culture. The difference between the duration of the lesions and recurrences of the disease, according to the species of *Malassezia*, is shown in Table 1.

The prevalence of pityriasis versicolor was greater in female patients and in young patients (mean age 31 years). These data are in agreement with reports in the literature that emphasize the higher frequency of this infection in young people due to the lipophilic characteristics of this type of fungus.² The most prevalent species of *Malassezia* in this sample population was *M. sympodialis* followed by *M. furfur* and *M. globosa*. These findings differ from those reported in a study carried out in the state of Goiás in Brazil in 2006 in which a prevalence of *M. furfur* of 77.8% was found. However, they are in

agreement with the findings of Framil et al., who also reported *M. sympodialis* as being the most prevalent species.⁷ Relapses are often reported by patients and cases of recurrence were found for almost all the species isolated; however, the number of relapses was not significantly different between the species of fungus. Framil et al. reported a predominance of *M. sympodialis* in patients who had experienced more relapses; nevertheless, this finding was not confirmed in the present study.⁸ The most prevalent sites of infection were the back, chest and abdomen in the case of most of the species and this finding is in agreement with the results published by Miranda et al. (2006), who also reported the back and chest as being the most common sites of the lesions.⁹ No statistically significant difference was found between the species as a function of gender, age or the duration of the lesions. To the best of our knowledge, there are no reports in the literature in which statistically significant differences were found between the clinical and demographic data and the species of *Malassezia*. Further studies with larger sample sizes may be required to clarify this issue. □

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