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Original

Prevalence of *Malassezia* species in pityriasis versicolor lesions in northeast Argentina

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ABSTRACT

Background: *Malassezia* species normally colonize the skin but they can change their saprophytic state and invade the stratum corneum as pathogens.

Aims: To determine the prevalence of *Malassezia* species isolated from patients with pityriasis versicolor (PV) and to analyse their distribution according to the location of the lesion on the body.

Methods: This study included 218 patients with PV and positive *Malassezia* cultures who resided in the city of Resistencia, a subtropical area located in northeast Argentina. Age, gender, and the body site of lesions were recorded. Strains were identified by PCR-RFLP.

Results: *Malassezia sympodialis* (37.7%) and *Malassezia globosa* (37.2%) were the most prevalent species isolated alone or in association with other *Malassezia* species in 82% of the patients. *Malassezia furfur* (21.3%) was the third most common species, followed by *Malassezia slooffiae* (1.7%), and *Malassezia restricta* (1.3%), which was found only in combination with *M. globosa* and *M. sympodialis*. *Malassezia dermatis* (0.4%) and *Malassezia pachydermatis* (0.4%) were each isolated once. None of the species affected a body site with statistical significance. Significant difference between genders according to age was found only in the 31–40-year-age group.

Conclusions: This study suggests that *M. sympodialis* and *M. globosa* represent the main species implicated in the pathogenicity of PV. *M. furfur* appears to be the third agent of importance in this geographical area. Statistical analyses showed none of the species was particularly associated with any one of the body sites.
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Prevalencia de especies de *Malassezia* en lesiones de pitiriasis versicolor en el nordeste argentino

Palabras clave:
Malassezia
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Ecología

RESUMEN

Antecedentes: Las especies de *Malassezia* colonizan normalmente la piel, pero ante ciertas condiciones pueden cambiar su estado saprofítico y transformarse en patógenas.

Objetivos: Estudiar la prevalencia de especies de *Malassezia* aisladas de pacientes con pitiriasis versicolor (PV) y su distribución de acuerdo al sitio anatómico de las lesiones.

Métodos: Este trabajo se realizó en la ciudad de Resistencia, ubicada en una región subtropical del nordeste de la Argentina. Se incluyeron 218 pacientes con PV y cultivo positivo para *Malassezia*. Edad, género y sitios anatómicos de las lesiones fueron registrados. Las cepas fueron identificadas por PCR-RFLP.

Resultados: *Malassezia sympodialis* (37,7%) y *Malassezia globosa* (37,2%) fueron las especies con mayor prevalencia, aisladas en el 82% de los pacientes, bien como agente único o en asociación con otras especies. *Malassezia furfur* (21,3%) se encontró en tercer lugar, seguida por *Malassezia slooffiae* (1,7%). *Malassezia restricta* (1,3%) se aisló solo en asociación con *M. globosa* y *M. sympodialis*. *Malassezia dermitis* (0,4%) y *Malassezia pachydermatis* (0,4%) fueron aisladas una sola vez. No se encontró una relación significativa entre las especies aisladas y los lugares anatómicos. Únicamente se encontraron diferencias significativas entre sexos (masculino y femenino) en el grupo de 31–40 años de edad.

Conclusiones: Estos resultados sugieren que *M. sympodialis* y *M. globosa* son las principales especies implicadas en la patogenicidad de la PV, sin predominio de ninguna de ellas. *M. furfur* aparece como el

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tercer agente en importancia en esta área geográfica. No se encuentra una relación estadísticamente significativa entre una determinada especie y algún sitio anatómico en particular.
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Malassezia species normally colonize the skin. For reasons currently unknown, these yeasts can change their saprophytic state and invade the stratum corneum as pathogens.[5,11,14,17,22]

Pityriasis versicolor (PV) is a chronic, benign skin disease that is generally asymptomatic. It occurs worldwide and is very common in tropical and temperate regions, where the major frequency of recurrence is observed.[14,16] It predominantly affects young adults of both genders, and manifests characteristic clinical lesions as slightly scaly macules that vary in colour from hypopigmented (white) to hyperpigmented (pink, tan to brown).[2,5,11,14,15]

The fungal nature of PV was recognized by Eichstedt in 1846 and in 1874 the round and oval budding yeast cells of the organisms were described by Louis Malassez.[15] Some years later, the genus *Malassezia* was created by Baillon with *Malassezia furfur* as the generic type species.[11,15,16] The variable and unstable morphology, and the strictly lipophilic nature that requires specialized media for growth, have complicated the study of this genus.[2,13,16,20]

Distinctive morphologic and physiologic features allow differentiating *Malassezia* from other yeast genera. However, as *Malassezia* species share several of these characteristics, a rapid and simple method to identify them does not exist. Identification schemes based on morphologic, physiologic, and biochemical features do not address the ambiguity between some species, nor do they permit differentiation of all currently described species.[1,3,9,18] In an effort to overcome the disadvantages of conventional methods, different molecular techniques have been developed. In the last decade, several PCR-based techniques for *Malassezia* species discrimination have been proposed, such as PCR with enzymatic restriction, randomly amplified polymorphic DNA (RADP), and pulsed field gel electrophoresis (PFGE).[1,10,18,20] Nevertheless, a specific, reliable, but also simple and inexpensive method for genotypic differentiation of all currently known *Malassezia* species is still not available.

Today, on the basis of morphology, ultrastructure, physiology, and molecular biology studies the genus *Malassezia* includes the following species: *M. furfur*, *Malassezia sympodialis*, *Malassezia globosa*, *Malassezia restricta*, *Malassezia obtusa*, *Malassezia slooffiae*, *Malassezia pachydermatis*, *Malassezia yamatoensis*, *Malassezia dermatis*, *Malassezia nana*, and *Malassezia japonica*.[12,19,23,25–27] A further species, *Malassezia equi*, has been described and tentatively named, but not fully characterized.[1] In 2007 *Malassezia caprae* and *Malassezia equina*, isolated from animals, were postulated as new species.[4]

The revision of the genus *Malassezia* opened up new questions about ecology and pathogenicity of *Malassezia* species. Many studies have looked at the geographical variation in species distribution; in addition, there are indications that the dominant species may vary at different body sites and in different conditions.[1,8,22] The aims of this study were to determine the prevalence of *Malassezia* species isolated from patients with PV and to analyse their distribution according to the skin lesion body sites.

Materials and methods

Between May 2006 and May 2008, skin samples obtained from patients with PV were studied at the Mycology Department of Instituto de Medicina Regional, Universidad Nacional del Nordeste, Resistencia, Argentina. All the patients included live in the city of Resistencia. Located in the northeast border of Argentina (27°55′S–58°22′O), it has a subtropical climate.[24]

Samples were scraped off with a sterile blade and subsequently were inoculated on modified Dixon's medium at 32 °C for 7 days.[13] In case of multiple lesions, all lesions were sampled and a record of the body site was made. If the same *Malassezia* species was isolated from different anatomical regions it was considered as a single isolate.

DNA extraction: Cellular lysis was performed by a boiling–thermic shock combination. One loopful of yeast was suspended in 80 µl of distilled water and boiled for 5 min at 100 °C, then frozen for 10 min at −70 °C, and finally boiled again for 5 min at 100 °C. The crude DNA suspension (extract) was stored at −20 °C until its analysis.

Strains were identified by PCR-RFLP. This technique allows the identification and differentiation of *M. furfur*, *M. sympodialis*, *M. globosa*, *M. restricta*, *M. obtusa*, *M. slooffiae*, *M. pachydermatis*, *M. yamatoensis*, *M. dermatis*, *M. nana*, and *M. japonica*.[21] Amplification was performed using generic primers, forward 5′-TAA CAA GGA TTC CCC TAG TA -3′ and reverse 5′-ATT ACG CCA GCA TCC TAA G -3′. The primers successfully amplified the target part of 26S rDNA from all tested *Malassezia* strains, providing a single PCR product of the expected size (approximately 580 bp).[21]

Amplified DNA products were subjected to further restriction fragment length polymorphism (RFLP) using separately *Cfo I* and *Mbo I*. *Cfo I* does not allow differentiation of *M. sympodialis* from *M. dermatis*. In order to determine the best specific digestion patterns, different restriction enzymes were analysed by CLC Free Workbench software (version 3.0.2 CLC bio A/S) for the high conserved 26S rDNA sequences of known *Malassezia* species (accession numbers: AJ249955, AJ249956, AJ249954, AB105862, AB105199, AJ249952, AJ249951, AB070365, AJ249953, AB125263, AJ249950 and CBS 9432). Based on this analysis, enzyme *Mbo I* was selected.

Amplified DNA products were subjected to restriction fragment length polymorphism (RFLP) using *Cfo I* (Promega) in a first identification step. Whenever the restriction pattern produced by *M. sympodialis* or *M. dermatis* appeared, additional *Mbo I* (Fermentas) was used for discrimination between these species.

CBS 7886 *M. globosa*, CBS 7019 *M. furfur*, CBS 7222 *M. sympodialis*, CBS 9169 *M. dermatis*, CBS 9432 *M. japonica*, CBS 10533 *M. pachydermatis*, CBS 7991*M. restricta*, CBS 9725 *M. yamatoensis*, CBS 7876 *M. obtuse*, and CBS 7956 *M. slooffiae* were included as reference strains.

Using the Chi-square test, PV relationships with age and gender were analysed. The Chi-square test was also used for all statistical analyses, and a P value of < 0.05 indicated statistical significance.

Results

Specimens were obtained from 218 PV patients including 109 males (age range: 7–61 years; median Z: 23 years) and 109 females (age range: 10–65 years; median Z: 23 years). The patients were categorized into 7 age groups (Table 1).

The highest prevalence of PV was observed between 11 and 40 years old (median Z: 24 years), but 42.6% of the cases were observed in the 21–30-year-old group (median Z: 26 years; Table 1).

Among the 218 patients, 56 had lesions in two different body sites and 6 patients in three (Table 2). A single agent was isolated in all cases of patients with multiple lesions. From these 218 patients, 239 strains were obtained with a distribution as follows: 200/218 (91.74%) PV patients had a single *Malassezia* species in their lesions, 15/218 (6.88%) had 2 species, and 3/218 (1.38%) had 3 species. Species that were isolated together in the same patient are displayed in Table 3.

The most frequent isolate was *M. sympodialis* [90/239 (37.7%)], followed by *M. globosa* [89/239 (37.2%)], *M. furfur* [51/239 (21.3%)], *M. slooffiae* [4/239 (1.7%)], *M. restricta* [3/239 (1.3%)], and finally *M. dermatis* [1/239 (0.4%)] and *M. pachydermatis* [1/239 (0.4%)] (Table 2).

Chi-square analysis for *M. globosa*, *M. sympodialis*, and *M. furfur* indicated that none of them were significantly associated with any of the body sites we distinguished, namely trunk, arms, neck, face, and groin (all P values > 0.05). The distributions of *Malassezia* species and gender relationships are presented in Table 4.

Discussion

The fungal origin of PV was recognized in the 19th century. The pathogenesis of this skin condition and the association of the new species with PV lesions are not yet well established.

In this study, *M. sympodialis* and *M. globosa* as agents of PV were significantly more common than other species; moreover, they were isolated in equal rates. In contrast with these results, others studies report one of these species as predominant in different geographical areas. Some of them report *M. globosa* as the predominant species related to PV[6,8,22] and others report *M. sympodialis* as the species with the highest rates of isolation from PV lesions.[16,17]

M. slooffiae and *M. restricta* were less isolated as previously reported.[7,16,22] It is interesting that *M. dermatis* was isolated from one patient and this is the first report of the isolation of this species in our region. This may be because it is the first time that a molecular method has been applied to *Malassezia* species identification in Argentina.

The isolation of *M. pachydermatis* from PV lesions is also reported for the first time in our region. As this patient had extensive lesions on trunk, arms, and neck, and *M. pachydermatis* was isolated from all of them, it is highly probable that it was the causative agent. This patient indicated he had close contact with

Table 3
Malassezia species isolated in several associations.

Malassezia species	*n*
M. sympodialis–M. furfur	4
M. sympodialis–M. globosa	5
M. globosa–M. furfur	4
M. furfur–M. dermatis	1
M. globosa–M. slooffiae	1
M. sympodialis–M. globosa–M. restricta	3
Total	18

Table 1
Number of patients and their distribution by age and gender.

Age group	*n*	M	F	(χ^2, *P* values)
1–10	4	3	1	0.31
11–20	54	27	27	1.00
21–30	93	49	44	0.49
31–40	32	9	23	0.02
41–50	21	11	10	0.81
51–60	12	9	3	0.08
Over 60	2	1	1	1.00
Total	218	109	109	

F: female; M: male.

Table 4
Malassezia species isolated by gender.

Malassezia species	M	F	(χ^2, *P* values)
M. sympodialis	49	41	0.27
M. globosa	37	54	0.019
M. furfur	28	21	0.26
M. slooffiae	2	2	1.00
M. restricta	2	1	0.56
M. dermatis	0	1	0.31
M. pachydermatis	1	0	0.31
Total	119	120	

F: female; M: male.

Table 2
Distribution of *Malassezia* species isolates according to body sites.

Body site		*Malassezia* species						
	n	*M. globosa*	*M. sympodialis*	*M. furfur*	*M. slooffiae*	*M. dermatis*	*M. restricta*	*M. pachydermatis*
Trunk	91	37	40	17	2	1		
Trunk–arms	21	4	11	8				
Trunk–arms–neck	6	3	2	1			1	1
Trunk–neck	9	4	3	2			2	
Trunk–face	4	2		3				
Trunk–groin	6	3	3	1				
Back	36	16	16	9				
Back–arms	2	1		1				
Back–face	6	1	2	3				
Arms	8	1	5	3				
Arms–neck	3	1	1		1			
FACE	12	6	3	3	1			
Face–arms	3	2	1					
Face–neck	2	1	1					
Neck	7	6	1					
Groin	2	1	1					
Total	218	89	90	51	4	1	3	1

Trunk: chest, back, and abdomen.

his dogs, which lived inside his house. Up to now *M. pachydermatis* has been considered only as a transient member of the human cutaneous biota[1]; therefore its association with PV deserves further investigation.

Anatomic sites from which more than one species was recovered included the back, trunk, neck, and groin. The PCR-RFLP methodology employed allowed the detection and characterization of *Malassezia* species mixtures, which otherwise are difficult to discriminate. Detection of mixtures would be important because *Malassezia* species could have different responses to antifungal agents.

As Mirhendi et al. indicated, *Cfo I* allows a clear discrimination of 9 *Malassezia* species but is not useful to differentiate *M. dermatis* and *M. sympodialis*. To differentiate these species they used *BstF51*[21]; however this enzyme is not available in our country. We were able, however, to distinguish these species using *Mbo I*.

PV lesions are more commonly seen on trunk and arms in a bilateral and asymmetrical distribution.[2,11,14,17,22] We observed a high frequency of patients with multiple lesions (28.4%) and lesions in unusual locations, such as neck, face, and especially the groin. Probably the high ambient temperature and humidity of our region encourage PV spread.[8]

Epidemiological studies point out a prevalence of some species in certain body sites and also their relationship with some pathology.[14,16,17,22] We did not observe differences in *Malassezia* species distribution according to the body sites. This might be related to the frequent presence of patients with extensive PV lesions. *M. restricta* is often associated with head, including scalp, neck, and face areas.[3,16] In this study, the three *M. restricta* isolations were always found in trunk and neck lesions in association with the more frequent species.

PV is a disease more commonly found among teenagers and young adults of both genders. Among children it is generally rare, and unusual in older adults, although cases are more common in tropical zones.[2,5,11,14,16,22] This agrees with the prevalence observed in this work, where 16% of cases were found in patients older than 41 years. It may be that subtropical climate factors of this region favour a broader age range of incidence for PV.

Significant difference between genders according to the patient's age group was found only in the 31–40-year-old group, with a higher frequency for female patients. This difference may be related to a higher frequency of women seeking treatment for aesthetic reasons.

The Chi-square test showed significant differences in species distribution according to sex only for *M. globosa*, which was more frequent in female patients. No special association with gender was obtained for the other species isolated.

Our results add to previously published epidemiological studies, all of which show geographical variations in *Malassezia* species distribution. This cannot be explained solely by differences in sampling techniques, culture medium, or identification procedures. Ethnic origin or/and geographical factors could contribute towards these differences.[1,8]

Since the taxonomic revision carried out by Guého et al.[12], a number of studies have evolved with the aim of elucidating the ecology and role of the different species in the pathological disorders associated with this group of yeasts. This work is a contribution to the knowledge of *Malassezia* genus ecology. Further studies will be necessary in order to explain the relationships of these species with the environment and with the associated pathologies.

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