

Clinical and epidemiological features of the genus *Malassezia* in Iran

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ABSTRACT

Background and Objectives: The genus *Malassezia* contains an expanding list of lipophilic yeasts involve in the etiology of various superficial fungal infections. Pityriasis versicolor (PV) is the most prevalent *Malassezia*-related infection distributed worldwide. In the present study, clinical and epidemiological features of the genus *Malassezia* are discussed with special focus on PV in Iran.

Materials and Methods: During June 2012 to April 2013, among 713 confirmed cases of fungal infections, 68 (9.5%) were diagnosed as PV by positive direct microscopy results in 20% potassium hydroxide (KOH) preparation of skin scrapings. All the specimens were cultured on modified Dixon agar and incubated at 32°C for 10 days. Identification of the isolated yeasts was carried out based on macro- and microscopic morphology, catalase test, utilization of Tweens, polyethoxylated castor oil (EL slant), and hydrolysis of esculin and utilization of Tween-60 (TE slant).

Results: Out of 68 skin scrapings, 55 (80.9%) yielded yeast colonies on mDixon's agar which were finally identified as *M. globosa* (36.36%), *M. pachydermatis* (29.08%), *M. furfur* (23.65%), *M. slooffiae* (7.28%) and *M. obtusa* (3.64%).

Conclusion: Results of the present study further indicate clinico-epidemiological importance of the genus *Malassezia* with growing importance of *M. pachydermatis* as a major species involve in the etiology of pityriasis versicolor. These findings are of major concern in management of *Malassezia*-related diseases.

Keywords: *Malassezia*, Pityriasis versicolor, Epidemiology, Species identification

INTRODUCTION

The genus *Malassezia* contains opportunistic yeast pathogens which reside on surface of normal skin of human and other warm-blooded vertebrates (1-4). These lipophilic yeasts are involved in the etiology of various human diseases including pityriasis versicolor (PV), seborrheic dermatitis, folliculitis and atopic dermatitis (5-8). PV is a chronic superficial fungal disease that is characterized by the appearance of round-to-oval lesions most

commonly found on the trunk and upper aspects of the arms. These lesions vary in color and can be hypopigmented (white) or hyperpigmented (pink, tan, brown or black) (9). It is common in young adults and usually presented as variable pigmented scaling maculae (10), and is diagnosed on the basis of its clinical appearance and the diagnosis can be confirmed by microscopy. A culture is essential for identification as to which of the lipophilic *Malassezia* species could be the causative organism in a particular case. More frequently Leeming and Notman agar or modified Dixon agar are used to culture *Malassezia* (11). *Malassezia* species belong to the basidiomycetous yeasts and is classified in the *Malasseziales* (*Ustilaginomycetes*, *Basidiomycota*) (12, 13). The taxonomy of *Malassezia* has undergone extensive revisions in the last 10 years. From the first description of Guillot and Gueho (1996) by

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introducing 7 species of *Malassezia*, i.e., *M. furfur*, *M. sympodialis*, *M. obtusa*, *M. globosa*, *M. restricta*, *M. slooffiae* and *M. pachydermatis* (14), seven new species have been added to the list including *M. dermatis*, *M. japonica*, *M. yamatoensis*, *M. nana*, *M. caprae*, *M. equina*, and *M. cuniculi* in last decade (15). Although all species are known to involve in the etiology of PV, their frequency and distribution is completely related to various environmental and host conditions such as geographic region, sex, age, etc (10, 16, 17). Several studies reported within the last decade, *M. furfur*, *M. globosa* and *M. sympodialis* are considered as the most species contributed in the etiology of PV and seborrheic dermatitis in Iran and other parts of the world (11, 18-25). Despite numerous available data are now exist about PV and other *Malassezia*-induced infections, many aspects of the genus regard to its clinical epidemiology and how it spread among populations are lacking. There is no comprehensive study considering all aspects of *Malassezia*-induced PV from new taxonomic criteria to prevalence of disease among infected populations from Iran. This study was carried out to respond major questions about the role of *Malassezia* species in the etiology of PV with special attention to their distribution in different clinical specimens in 2019 cases suspected to fungal infections during nine months period.

MATERIALS AND METHODS

Patients. Since June 2012 to April 2013, 68 cases suspected to pityriasis versicolor referred to Department of Mycology at the Pasteur Institute of Iran were included in this study. All the procedures were in accordance with the ethical standards of Ethics Committee of the Pasteur Institute of Iran which is compatible with the Helsinki Declaration 1975. Clinical samples (skin scrapings) were examined by direct microscopy and then cultured on general and specific culture media.

Direct microscopic examination. Skin scrapings were collected from 68 patients suspected to PV, and mounted in 20% KOH for direct microscopic examination. They were assessed by observing morphological features of *Malassezia* including budding yeast cells and/or hyphae under $\times 40$ objective lens.

Isolation and identification of *Malassezia* species. All 68 specimens were cultured on modified Dixon agar (3.6% malt extract agar, 0.6% mycological peptone, 2% desiccated ox-bile, 0.2% glycerol, 1% Tween 40, 0.2% oleic acid, 1.2% agar, 0.005% chloramphenicol and 0.05% cycloheximide, pH 6.0) and incubated at 32°C for 10 days. Identification was performed based on macro- and microscopic morphology on mDixon's agar, the ability for growth on Sabouraud dextrose agar, catalase test, utilization of Tweens, polyethoxylated castor oil (EL slant), and hydrolysis of esculin and utilization of Tween 60 (9, 26, 27).

Malassezia pachydermatis can grow on Sabouraud dextrose agar (SDA, E-Merck, Germany) which was composed (per liter) 10 g of mycological peptone, 40 g of glucose and 15 g of agar (27, 28). For confirmation of identity of this species, colonies grown on subcultured SDA from mDixon's agar were transferred to SDA plates several times.

Catalase reaction. Presence of catalase was determined by using a drop of hydrogen peroxide (3% solution) and production of gas bubbles was considered as a positive reaction. Lack of catalase activity is an acceptable key identification of *M. restricta* (28).

Tween assimilation test. For each isolate the ability to utilize individual Tweens was examined by the following procedure. *Malassezia* yeasts suspensions were prepared in 5 ml distilled water which was adjusted to 0.5 McFarland turbidity. Two ml of suspension was poured into the plate involved 16 ml Sabouraud dextrose agar (SDA) supplemented with 0.05% cycloheximide and 0.005% chloramphenicol. Four holes were punched in the agar by the means with 2mm diameter and filled with 10 μ l of each of Tweens 20, 40, 60 and 80 respectively. The agar plates incubated at 35°C for a week and were examined each day for the existence of any growth around the wells that contained Tween compounds (29).

Utilization of polyethoxylated castor oil (EL slant). Cremophor EL which can indicate assimilation of castor oil was composed of (per liter) 65 g of SDA and 10 ml of Cremophor EL (26, 27). EL slants were used to determine the ability to utilize polyethoxylated castor oil. Only some colony yeasts can grow and others cannot (26).

Table 1. Results of direct microscopic examination (DME) of *Malassezia* yeast cells based on growth on mDixon's agar

Pityriasis versicolor	DME	Culture
Positive	68(100)	55(81)
Negative	0(0)	13(19)
Total	68(100)	68(100)

Values are given as n (%).

Hydrolysis of esculin and utilization of Tween-60 (TE slant). TE slant contained (per liter) 10 g of peptone, 10 g of glucose, 2 g of Yeast extract, 5 ml of Tween-60, 0.5 g of ferric ammonium citrate, 1 g of esculin and 15 g of agar (26, 27). Some strains can produce a black zone due to esculin hydrolysis products and ferrous iron on TE slants. Some are able to grow on TE slants with no production of black zone (26).

RESULTS

Out of 68 cases (9.5%) suspected to pityriasis versicolor, 41 (60.3%) were male and 27 (39.7%) were female. All the 68 skin scrapings demonstrated pseudohyphae and more than 1-3 budding yeast cells in each microscopic field in direct microscopic examination. Fifty-five (80.9%) of the skin scrapings yielded yeast colonies after culturing on mDixon's agar (Table 1).

This indicates that direct microscopic examination is essential for diagnosis of PV as the first step. Among 55 isolated *Malassezia* species, twenty of the isolates were belonged to *M. globosa* (36.36%), *M. pachydermatis* (n= 16; 29.08%), *M. furfur* (n=13; 23.65%), *M. slooffiae* (n=4; 7.28%) and *M. obtusa* (n=2; 3.64%). The mean age of the patients was 30 years. The highest prevalence of PV was seen

in patients with 20-29 years. The higher rate of infection was reported in male (60.3%) than that of female (39.7%) (Table 2). Distribution of *Malassezia* species in different body sites is shown in Table 3.

The most common sites were the trunk 12 (21.82%), groin 11 (20%) and face 10 (18.18%). *M. globosa* had the most frequency on groin, *M. pachydermatis* on face and *M. furfur* on trunk. The phenotypic and biochemical characteristics of *Malassezia* species are shown in Table 4. Sixteen out of 55 species (29.08%) grew on the lipid free culture medium (SDA) which all was identified as of *M. pachydermatis* as the only non-lipid dependent species in the genus. *M. restricta* as the only catalase negative species was not isolated in the present study. Examining the ability to utilize polyethoxylated castor oil in EL slants showed no growth for *M. globosa*, *M. obtusa* and *M. slooffiae*. Sixteen (29.08%) of *M. pachydermatis* and 13 (23.65%) of *M. furfur* showed production of a black zone around the colonies due to esculin hydrolysis products and ferrous iron in TE slant. *M. globosa* could not grow and produce a black zone in TE slant. *M. slooffiae* isolates grew in TE slant without producing a black zone. *M. obtusa* isolates produced a black zone in TE slant without the ability for growth on this medium. The Tween diffusion test allowed differentiation of the most *Malassezia* species in this study. *M. furfur* utilized the four individual Tweens.

Table 2. Distribution of *Malassezia* species in different age groups of patients with pityriasis versicolor in both sexes.

Age groups (year)	Malassezia species [number (%)]	Male	Female
0-9	2 (2.9)	1 (2.4)	1 (3.7)
10-19	14 (20.6)	6 (14.6)	8 (29.6)
20-29	22 (32.3)	15 (36.6)	7 (25.9)
30-39	12 (17.6)	10 (24.4)	2 (7.4)
40-49	9 (13.2)	4 (9.8)	5 (18.5)
50-59	9 (13.2)	5 (12.2)	4 (14.8)
Total	68 (100)	41 (100)	27 (100)

Table 3. Distribution of *Malassezia* species in different body parts in patients with pityriasis versicolor

Species	Involved body site [number (%)]									Total
	Face	Trunk	Neck	Abdomen	Groin	Forearm	Thorax	Hair & Scalp	Waist	
<i>M. globosa</i>	3 (30)	3 (25)	2 (28.6)	3 (75)	5 (9.1)	0 (0)	1 (20)	1 (50)	2 (66.7)	20 (36.4)
<i>M. pachydermatis</i>	5 (50)	3 (25)	2 (28.6)	0 (0)	3 (5.4)	0 (0)	3 (60)	0 (0)	0 (0)	16 (29.1)
<i>M. furfur</i>	2 (20)	4 (33.3)	2 (28.6)	0 (0)	2 (3.6)	1 (100)	1 (20)	0 (0)	1 (33.3)	13 (23.6)
<i>M. slooffiae</i>	0 (0)	0 (0)	1 (14.3)	1 (25)	1 (1.8)	0 (0)	0 (0)	1 (50)	0 (0)	4 (7.3)
<i>M. obtusa</i>	0 (0)	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.6)
Total	10 (100)	12 (100)	7 (100)	4 (100)	11(100)	1 (100)	5 (100)	2 (100)	3 (100)	55 (100)

The growth of *M. pachydermatis* was inhibited by high concentration of Tween 20. The growth of *M. slooffiae* was inhibited with high concentrations of Tween 80. *M. globosa* and *M. obtusa* could not able to utilize any of four Tweens.

DISCUSSION

Pityriasis versicolor is a very complex disease and many aspects of disease are obscure despite the old history of occurrence and identification. The correlation of disease with environmental and host factors have not been clearly described. Regarding

to the host, it believes that frequency of *Malassezia* yeasts recovery depends on various factors such as age, sex, body sites and other various internal and external factors and the result varies according to the difference in techniques of identification (29). Today the genus of *Malassezia* contains 14 lipophilic species that have been isolated from healthy and diseased human and animal skin (30).

In the present study, among five *Malassezia* species contributed in PV, *M. globosa* was the most prevalent species followed by *M. pachydermatis*. It is considered that *M. globosa* is a dominant causative agent of PV in many parts of the world especially in

Table 4. Biological and biochemical characteristics of *Malassezia* species isolated in the present study

Species (No.)	Cell morphology	Catalase reaction	Growth on mDixon's at 32C°	Growth on SDA at 32C°	Tween assimilation				Growth on EL slant	Growth on TE slant
					Tween 20	Tween 40	Tween 60	Tween80		
<i>M. globosa</i> (20)	Globose	+	+	-	-	-	-	-	No growth	No growth and no change
<i>M. pachydermatis</i> (16)	Ellipsoidal	+	+	+	-	+	+	+	Growth	Growth and produced a black zone
<i>M. furfur</i> (13)	Globose, Ellipsoidal	+	+	-	+	+	+	+	Growth	Growth and produced a black zone
<i>M. slooffiae</i> (4)	Ellipsoidal, cylindrical	+	+	-	+	+	+	-	No growth	Growth and no change
<i>M. obtusa</i> (2)	Ellipsoidal, cylindrical	+	+	-	-	-	-	-	No growth	No growth but produced a black zone

temperate regions such as Iran. Our finding for *M. globosa* is in accordance with surveys carried out in Iran and other parts of the world including Greece, Italy, Turkey and India (11, 18, 22-25, 31-34). It may be due to the fact that *M. globosa* is a species with high levels of esterase and lipase enzymes which are contributed in fungal pathogenicity (35). In contrast, Gupta *et al.* indicated *M. sympodialis* was the predominant isolated species followed by *M. globosa* in Canada (36). Also, in a study from Indonesia, Krisanty *et al.* reported that *M. furfur* following by *M. sympodialis* and *M. globosa* were the most species isolated (37). In the present study, *M. globosa* was mainly isolated from groin. Hedayati *et al.* (18) and Gupta *et al.* (36) reported *M. globosa* mainly from face and scalp and *M. furfur* from trunk.

According to the clinical sign or anatomical sites of lesions, *M. globosa* was found more in groins of men in the age group of 20-29 years. *Malassezia* yeasts can be found in wrinkled sites of body and existence of it depends on some factors such as humidity, amount and composition of skin lipids. It seems that the higher prevalence of *Malassezia* yeasts in men can be related to their higher involvement in social activities. The frequency of *Malassezia* yeasts increased with age which can be attributed to the fact that sebaceous secretion reaches to a peak at adolescents (38). Kwon *et al.* (39) reported a higher frequency of *Malassezia* in adults rather than adolescents.

In present study, familiar history of all patients referred to medical mycology laboratory was surveyed carefully. It is of noteworthy that all the examined patients revealed negative evidence to background disease, antibiotic long term usage and pet contact.

M. pachydermatis is the only lipid-dependent species which can grow on SDA and it is considered as a zoophilic species. It has been reported as an unusual species involved in the etiology of PV in the world. Results from Rasi *et al.* reported a total prevalence of 7.2% for this species with no apparent source of species (11), while we interestingly reported a high prevalence of 29.1% as the second species to *M. globosa* in PV. This species was isolated more from the face of patients. The original source of *M. pachydermatis* was unclear in our survey. Nowadays people are more inclined to look after pets at home in Iran than before. In some cases the sources of human infections addicted to *M.*

pachydermatis have been traced to pet dog's owners. It is explained mechanical transfer of *M. pachydermatis* from the skin of dogs to the healthy skin of humans occurs commonly. Since *M. pachydermatis* has been reported from normal skin of <1% of normal individuals, direct or indirect transmission from person to person is also possible (40).

Direct microscopic examination of *Malassezia* yeasts plays a main role to identify PV. In our survey, 100% of the direct microscopic examinations of the specimens were positive and all showed short hyphae and yeast. In addition, 100% of patients with PV yielded more than 1-3 yeast cells per hpf. It has been shown that increasing the amount of lipids in the skin of patients with PV can be related to elevating in the numbers of *Malassezia* yeasts (41, 42). Isolation in culture is essential for identifying *Malassezia* species. Fifty-five (81%) of the specimens which were positive in direct microscopy were yielded yeast colonies on mDixon's agar. It may be due to the loss of ability of some *Malassezia* yeasts to grow on culture media due to previous encountering with antifungals used by patients before admitting for a mycological examination.

In conclusion, our results suggested that *Malassezia*-related infections can be considered as important superficial fungal diseases in Tehran with an approximate frequency of 10-15% as indicated here and in another comprehensive study in our laboratory during 2006 to 2009 (43). Although *M. globosa* was reported as the major etiologic agent of PV, high prevalence of *M. pachydermatis* in the present work indicates a possible shift in the epidemiology of *Malassezia* infections which may be due to increasing of pet animals in indoor environments. So, further studies on the prevalence and distribution of *Malassezia* species with special attention to the predisposing factors and underlying diseases facilitating the onset of infection are recommended.

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