Acta Dermatovenerol Croat 2016;24(4):274-281 CLINICAL ARTICLE

Distribution of *Malassezia* Species in Patients with Different Dermatological Disorders and Healthy Individuals

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Received: April 20, 2015
Accepted: October 5, 2016

ABSTRACT There are differences with respect to the commonly isolated *Malassezia* species, not only between healthy individuals and the patients with various skin diseases, but also between different countries. We investigated the species composition of *Malassezia* microflora on the skin of patients with *Malassezia*-associated diseases and of healthy subjects (HS). Two hundred and fifty skin scrapings from patients with pityriasis versicolor (PV), seborrheic dermatitis (SD), atopic dermatitis (AD), psoriasis (PS), and healthy subjects (HS), fifty each, were inoculated into Sabouraud dextrose agar and into modified Dixon agar and identified using conventional culture-based methods. In PV and PS lesions, the most common species was *M. globosa* (62% and 52%, respectively), while *M. restricta* was predominant in SD lesions (28%). *M. sympodialis* was the most common species recovered from AD (52%) and healthy trunk skin (30%). Fewer cultures were positive for *Malassezia* growth in patients with AD than in patients with other skin conditions, and even in controls. Our data are in agreement with other studies and suggest that the pathogenic species of PV is *M. globosa*. The evidence that any given species is clinically important in the pathogenicity of SD, AD and PS is still lacking.

KEY WORDS: *Malassezia* species, identification, healthy skin, dermatological disorders

INTRODUCTION

Yeasts of the genus *Malassezia* are now considered identical with those previously named *Pityrosporum,* which are members of the normal cutaneous microflora but are also associated with several dermatological disorders and even systemic infection (1).

Currently, *Malassezia* species have been classified into at least 14 species, eight of which have been isolated from human skin: *M. furfur, M. pachydermatis, M. sympodialis, M. slooffiae, M. globosa, M. obtusa, M. restricta, M. dermatis*, *M. japonica*, and *M. yamotoensis* (2).

Colonizing the seborrheic areas of the skin, a commensal status of *Malassezia* cannot be clearly distinguished from the pathogenic stage, with the exception of pityriasis versicolor (PV) in which *Malassezia* yeasts can be seen under the light microscope in its mycelial phase (3,4). In the case of the other dermatoses such as seborrheic dermatitis (SD), *Malassezia* folliculitis, atopic dermatitis (AD), confluent and reticulate papillomatosis, and psoriasis (PS), the pathogenic role of *Malassezia* yeasts remains less clear; transition

of the yeast cells to their pathogenic hyphal form cannot be clearly demonstrated (1).

The spectrum of fungal species in the human skin has been explored using conventional, culture-based methods and molecular techniques, and variable results have been reported from different geographical regions (5-7). Identification of *Malassezia* yeasts to a species level is of no diagnostic value in skin diseases, as the same species form an integral part of normal cutaneous microflora in humans. However, it is of great importance to determine which species are implicated in certain skin diseases and whether there is variation in the distribution of the yeasts with clinical data, body site, origin of the population, etc. In addition, *Malassezia* species are susceptible to a wide range of topical and systemic antifungal therapy that may be important for the selection of sensitive drugs (5,6).

This study was undertaken with following objectives: (1) to investigate the prevalence of *Malassezia* species on the skin of patients with PV, SD, AD, PS, and of healthy subjects (HS) using a combination of physiological and biochemical tests; (2) to determine whether the composition of *Malassezia* species differs between patients with various cutaneous dermatoses and HS; (3) to review the literature focusing on the most recent studies.

PATIENTS AND METHODS

Patients

Two hundred patients with different *Malassezia*-associated diseases: PV (24 women and 26 men aged 12-66 years, mean 44), SD (22 women and 28 men aged 7-72 years, mean 46), AD (25 women and 25 men aged 2-60 years, mean 28) and PS (18 women and 32 men aged 7-72 years, mean 52), fifty each, were selected for the study. Normal subjects consisted of 50 healthy volunteers (25 women and 25 men aged 2-80 years, mean 55) with normal-appearing skin and without any evidence of a dermatosis.

The diagnosis of PV, SD, and PS was based on clinical features, and cases of PS were additionally confirmed by histopathological analysis. The diagnosis of AD was made according to the Hanifin-Rajka criteria (8).

Only those subjects who had not used any topical and oral treatment or ultraviolet phototherapy during the previous two months were included in the study. No concomitant diseases were registered. The patients were instructed not to take a shower or use emollients on the day of examination.

All participants gave their informed consent according to the requirements of the Institutional Ethics Committee.

Samples

Skin scales were scraped with a sterile blade: in patients with PV and AD from the lesional skin of the upper part of the trunk, in patients with SD and PS from the lesional skin of the scalp, and in HS from the healthy skin from both sites (scalp and trunk).

Collected samples were processed to the mycological laboratory and inoculated within the same day into Sabouraud dextrose agar (SDA) and into modified Dixon agar consisting of 3.6% malt extract, 0.6% mycological peptone, 2.0% desiccated ox bile (Sigma Chemical Co. Ltd, Dorset, UK), 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.05% cycloheximide, and 1.2% agar pH 6.0. The medium was always used within one week of preparation and the cultures were inoculated at 32°C for seven days.

Identification of Malassezia yeasts

Malassezia species were identified according to their macroscopic and microscopic features and

Table 1. *Malassezia* species isolated from patients with pityriasis versicolor (PV), seborrheic dermatitis (SD), atopic dermatitis (AD), and psoriasis (PS) and from healthy subjects (HS)

species	PV (n,%) trunk	SD (n,%) scalp	AD (n,%) trunk	PS (n,%) scalp	HS (n,%) trunk	HS (n,%) scalp
M. globosa	31 (62)	9 (18)	3 (6)	26 (52)	10 (20)	8 (16)
M. sympodialis	8 (16)	6 (12)	26 (52)	2 (4)	15 (30)	4 (8)
M. restricta	0	14 (28)	1 (2)	5 (10)	1 (2)	16 (32)
M. furfur	5 (10)	6 (12)	2 (4)	4 (8)	7 (14)	1 (2)
M. obtusa	4 (8)	0	0	0	0	1 (2)
M. slooffiae	2 (4)	8 (16)	0	9 (18)	1 (2)	5 (10)
M. pachydermatis	0	1 (2)	0	0	0	0
Positive	100 (100)	44 (88)	31 (62)	46 (92)	34 (68)	35 (70)
TOTAL	50	50	50	50	50	50

PV: pityriasis versicolor; SD: seborrheic dermatitis; AD: atopic dermatitis, PS: psoriasis, HS: healthy subjects

physiological characteristics, as previously *described in detail (*9).

Statistics

The data of the patients and healthy controls were included in a new database constructed using the SPSS statistical software package. Chi-squared test with Yates' correction for a small sample size was performed for evaluation of the differences in proportions. Two-tailed *P*-values less than 0.05 were considered statistically significant.

RESULTS

Mixed cultures were not observed. Only one *Malassezia* species was recovered from each of the samples.

All samples from PV lesions gave positive cultures. *M. globosa* was the predominant isolate (62%), followed in frequency by *M. sympodialis* (16%), *M. furfur* (10%), *M. obtusa* (8%), and *M. slooffiae* (4%).

In case of patients with SD, culture was positive in 88% samples. *M. restricta* (28%) was the most prevalent isolate, and the prevalence of other species was 18% for *M. globosa,* 16% for *M. slooffiae*, and 12% for *M. sympodialis* and *M. furfur* each. One isolate of *M. pachydermatis* was the only isolate which grew on SDA medium being lipid non-dependent.

In patients with AD, *Malassezia* yeasts were isolated in 64% cases. *M. sympodialis* was the dominant species (52%), while other species were less frequently isolated: *M. globosa* (6%), *M. furfur* (4%), and *M. restricta* (2%).

Malassezia yeasts were found in 92% samples taken from scalp skin of patients with psoriasis. The most frequently isolated species was *M. globosa*, found in more than a half of the samples (52%), followed by *M. slooffiae* (18%), *M. restricta* (10%), *M. furfur* (8%), and *M. sympodialis* (4%).

The results of culture obtained from healthy trunk skin were positive for *Malassezia* yeasts in 68% cases. The predominant species was *M. sympodialis* found in 30% of the patients, and the prevalence of other species was 20% for *M. globosa* and 14% for *M. furfur.* Both *M. restricta* and *M. slooffiae* were cultured in 2% of the samples. *Malassezia* yeasts were found in 70% samples obtained from healthy scalp skin. *M. restricta* was the dominant species (32%), followed by *M. globosa* (16%), *M. slooffiae* (10%), and *M. sympodialis* (8%). Both *M. furfur* and *M. obtusa* were identified in a single case (2%).

Table 2. Summary of the predominat *Malassezia* species isolated from healthy and diseased skin from different countries

Condition	Predominant species	Country (Reference)
Healthy skin	*M. sympodialis*	Spain (3), Poland (5), India (24), Sweden (25), Canada (26)
	M. globosa	Japan (18,22), Iran (19,21), Tunis (20)
	M. restricta	Japan (14), Korea (15,23)
Pityriasis versicolor	*M. globosa*	Spain (3,4), Japan (18,30), Iran (19), Tunis (20,32), India (24,28,34), Greece (29), Bosnia and Herzegovina (31), Sudan (33), Italy (35), Israel (36), Turkey (37)
	M. sympodialis	Canada (26,38), Argentina (39), Brasil (40)
	M. restricta	Japan (30)
	M. furfur	Indonesia (41)
Seborrheic dermatitis	*M. globosa*	Japan (18,22), Canada (26), Greece (29), Serbia (45), Iran (47,48), Argentina (49), China (50)
	M. restricta	Spain (3), Korea (46), USA (51)
	M. obtusa	Sweden (25), Poland (52)
	M. sympodialis	Sweeden (25)
	M. furfur	Japan (18)
Atopic dermatitis	*M. sympodialis*	Poland (5), Sweden (25), Canada (26), Korea (56)
	M. globosa	Japan (14,22,57)
	M. furfur	Japan (18)
Psoriasis	*M. globosa*	Iran (21), Canada (26), Bosnia and Herzegovina (63), Spain (64)
	M. restricta	Japan (67,68)
	M. furfur	Poland (5), India (65)
	M. sympodialis	Mexico (66)

Malassezia species isolated from patients with PV, SD, AD, and PS and from HS are shown in Table 1.

A statistically significant difference in isolated species was found in cases of *M. globosa* (recovered more frequently in patients with PV) ($P<0.001$), *M. sympodialis* (predominant species in patients with AD) ($P<0.001$), *M. restricta* (most commonly isolated from scalps in patients with SD and HS) ($p<0.001$). In patients with PS and SD, *M. slooffiae* was found more frequently than in other study groups ($P=0.04$). Regarding other species, the difference was not significant (*M. furfur*: $P=0.266$, *M. obtusa*: $P=0.18$) or the sample was insufficient (*M. pachydermatis*).

Fewer cultures were positive for *Malassezia* growth in patients with AD (62%) than in patients with PV (100%), PS (92%), SD (88%), or from healthy scalp (70%) or trunk skin (68%) ($P<0.001$).

DISCUSSION

With the recent enlargement of the genus, new questions have been raised about whether there exists a relationship between particular *Malassezia* species and various skin conditions; conflicting results have emerged, with different species predominating in different countries (Table 2).

Malassezia yeasts are unique among the fungal kingdom as the only species to form part of normal human cutaneous commensal flora. A number of researchers have conducted studies of *Malassezia* colonization of healthy skin (10-24). In general, it seems that the most common species cultured from healthy trunk skin are *M. globosa* and *M. sympodialis* (3,15,18-26), while *M. restricta* is the most frequently isolated species from the scalp (14-16).

In line with the majority of studies, we found that the predominant species on normal trunk skin was *M. sympodialis* isolated in 30% of cases. This species emerges as the predominant species on healthy skin, especially on the trunk, where it can be recovered in great numbers in more than 40% of individuals (3,5,24,25). In our study, *M. globosa* is a less common species found in 20% of healthy individuals which agrees with the studies of Kaur *et al.* (24) and Gupta *et al.* (26). In contrast to healthy trunk skin, *M. sympodialis* was recovered less frequently from the scalp skin of same subjects (8%), whereas *M. restricta* was the commonest species (32%). This species is isolated regularly from the scalp and face of patients with SD (3,22).

PV is the only human skin disease in which the causative role of *Malassezia* yeasts is fully established (3,4,24). Several groups have published studies examining the mycology of PV. Overall, the most common cultured species was *M. globosa*, isolated from between 49% and 97% of patients (3,4,18-20,24,27-37). In this survey, we found *M. globosa* to be the most common species isolated in culture from 62% of the cases. *M. sympodialis,* the second most common agent, has been isolated more frequently (16%) than in other studies, reporting frequencies between 4-13% (18-20,27-29). However, other studies from Canada, Argentina, and Brazil have reported *M. sympodialis* to be the predominant isolate found at frequencies of 59%, 38%, and 30%, respectively (26,38-40). *M. furfur*, on the other hand, was isolated less frequently (10%) than by many researchers who reported it as the second or third species (19,20,26,28,34,38), or even the first causative agent in a Indonesian culture-based study (41) .

It has previously been stated that among *Malassezia* species, *M. globosa* has the highest lipase activity (27). Lipolytic enzymes such as esterases and lipases may play an important role in the growth and pathogenicity of this species and provide an explanation of *M. globosa* as the most important pathogenic species in lesional skin of PV (42). The presence of this species in its yeast phase in diseased and even in healthy skin indicates that local factors (humidity, sweat, heat), together with some degree of idiosyncratic individual predisposition, are responsible for the transformation from yeast to the mycelial form and development of clinical lesions (4,19,38).

SD is a chronic, multifactorial disease characterized by *Malassezia* colonization, impaired barrier function, and subsequent inflammation. Numerous studies have supported a direct causal link between *Malassezia* yeasts and SD, focusing either on the microbiology of the condition or on the therapeutic efficacy of antifungal drugs (43,44).

Most of these studies demonstrate geographical variations in the rate of the isolated species, although a correlation between yeast density and severity of SD has been reported (45). In our study, 88% of examined patients had positive culture for *Malassezia* species, which is in agreement with the results of Arsic *et al.* (45) and Lee at al. (46) who showed that the rate of recovery of *Malassezia* yeasts was nearly the same: 87% and 85%, respectively. However, in a study conducted in south Iran the prevalence in patients with SD was very low at 24.5%, likely due to different climate in that part of the country, which is very dry (47).

In Japan (22), Canada (26), Greece (29), Serbia (45), Iran (47,48), Argentina (49), and China (50), *M. globosa* was most commonly identified, whereas *M. restricta* was predominant in patients with SD in Spain (3), Korea (46), and the USA (51). In Sweden (25) and in

Eastern Europe (52), however, skin lesions are more frequently colonized with *M. obtusa*, a species that was sporadically identified in previous studies from diseased (19,22,24-26,31,37,40,41) and healthy skin (22,24,25,46).

We identified *M. restricta* as the prevalent species from scalps in patients with SD (28%), while *M. globosa* was the second most frequently isolated species (18%). Lee *et al.* (46) and Gemmer *et al.* (51) also found that *M. restricta* was the predominant species associated with the scalp, at a frequency of 64% and 47%. However, both studies were performed using a molecular non-culture-dependent method.

Malassezia species are considered to be one of the factors that exacerbate AD, especially in a subset of patients with head and neck type of AD (53). The evidence that there is a connection with *Malassezia* is based on the observation that patients with AD respond to antifungal therapy (54) and that there is a high prevalence of a specific IgE antibody to *Malassezia* antigens in affected patients (55). In this study, the dominant species was also *M. sympodialis*, isolated from more than half the positive cases. Our result of the distribution of the *Malassezia* species was almost the same as those published in the aforementioned studies (5,25,26) , while *M. furfur* and *M. globosa* were the dominant species in Japan (14,18). Similarly, *M. sympodialis* was the dominant species among Korean patients with AD, yet the isolation rate was low (16.3%) (56).

Conversely, studies that used culture-independent methods showed *M. globosa* and *M. restricta* as the predominant species among the Japanese (14,22,57).They were detected at frequencies ranging from 87.5% to 100%, while *M. sympodialis* was the third most frequently isolates species, at 40.6% and 58.3% (14,22) .

The recovery rate of *Malassezia* in our study (62%) was lower than that obtained from patients from other study groups (PV, SD, PS) or from healthy controls, which is in agreement with some other studies (25,26,56). One reason for this may be disrupted skin barrier function in patients with AD, which reduces the amount of lipids to support the growth of yeasts (58). Another explanation for the low positive rate of isolated cultures may be the antifungal activity of the mediators and/or inflammatory cells present in AD lesions (26). It was also confirmed that the prevalence of positive *Malassezia* cultures was not correlated with the severity of AD (56) .

The involvement of *Malassezia* yeasts in PS is supported by the favorable effect of both oral and topical ketoconazole, which results in reduction of yeasts (59,60). Recent reports have indicated that *Malassezia* yeasts cause exacerbation of PS by triggering the release of cytokines, in particular IL-8 through a Toll-like receptor 2-mediated pathway (61), activating complement and recruiting neutrophils as well as inducing immune cell migration to the dermis, potentially enhancing inflammation (62). However, it still remains unclear whether these microorganisms are able to initiate the development of psoriasis lesions.

Studies to determine which species of *Malassezia* might be involved in PS have yielded divergent results, with *M. globosa* (21,26,63,64), *M. furfur* (5,65), and *M. sympodialis* (5,66) all found to be predominant species in psoriatic scale samples. The frequencies of the major isolate varied between 38% and 71%. However, in culture-independent studies, *M. restricta* was reported as the main species in patients with PS isolated at the high frequencies of 96% and 92%, respectively (67,68). We found that the predominant species in PS lesions from the scalps of our patients was *M. globosa*, isolated in 52% of cases. *M. slooffiae*, first isolated from animals, is considered to be very rare on healthy skin (15,18,22,24,25) and only infrequently isolated in the cases of PV (19,20,22,26,27,31,32,40,41), AD (18,22,25,26,56), SD (22,25, 46 49,66), and PS (26). However, it was the second most frequent species in our study (18%). The remaining three species, *M. restricta, M. furfur,* and *M. sympodialis,* were recovered at lower frequencies as a part of normal human cutaneous flora (1).

The discrepancies between the studies, as discussed above, in the frequencies of *Malassezia* species isolated from different dermal conditions may be attributed to several factors, including different sampling techniques (swabbing, scraping, contact plates, tape striping), different culture media (modified Dixon agar, Leeming and Notman agar), different isolation techniques (culture-dependent and molecular methods) and the various growth characteristics of each species. Geographical and ethnic origin, clinical and demographic characteristics, and even lifestyle habits of the subjects may contribute to the differences observed in the prevalence and species composition of *Malassezia* species.

The significant variation of the distribution of *Malassezia* species was especially apparent in the case of PV, in which *M. globosa* was reported as the causative agent in most of the studies. However, some subsequent findings have shown widespread distribution of *Malassezia* species on healthy and diseased skin (33,36), thus failing to establish the existence of a pathogenic species not only in PV but also for the other *Malassezia*-associated diseases.

CONCLUSION

Our results are in agreement with the majority of studies worldwide, which have identified *M. globosa* as the predominant, if not only, species involved in the etiology of PV. This species was also the most common in PS; however, it was isolated less frequently than in patients with PV. Patients with AD presented with *M. sympodialis* more frequently than any other study group. *M. restricta* and *M. slooffiae* showed a preference for the scalp. We found fewer individuals with positive culture in the AD group than in other patients groups or in HS.

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