

Identification of *Malassezia* Species Isolated From Patients With Pityriasis Versicolor in Sari, Iran, 2012

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Received: October 20, 2012; Revised: January 11, 2013; Accepted: February 23, 2013

Background: The genus *Malassezia* is part of the normal mycota of the skin of humans and other warm-blooded animals as etiological agents of pityriasis versicolor.

Objectives: Several species from the *Malassezia* genus are known based on morphological, biochemical and molecular approaches. Therefore, the aim of this study was to determine the distribution of *Malassezia* species in patients with pityriasis versicolor based on morphological, physiological and biochemical criteria in Sari, Iran.

Materials and Methods: In total, among 134 patients clinically suspected of having pityriasis versicolor, attending the Department of Mycology, Boali Sina Hospital and Referral Laboratory of the Mazandaran University of Medical Sciences, 116/134 (86.5%) positive patients for *Malassezia* elements, namely, yeast cells and short hyphae in microscopic examination, were included in the study. All 116 samples were inoculated on plates containing modified Leeming and Notman agar medium and identified at species level based on mycological criteria.

Results: However, only 100/116 (86.2%) or 100/134 (74.6%) of the patients showed *Malassezia* spp in culture. *Malassezia. globosa* (54%) was the most commonly isolated species followed by *M. furfur* (32%), *M. slooffiae* (6%), *M. restricta* (6%) and *M. sympodialis* (2%). Mixed *Malassezia* species were not identified.

Conclusions: *M. globosa* was found to be the predominant pityriasis versicolor isolate in Sari, Iran. Since *Malassezia* species show different responses in their antifungal therapy, thus correct identification of *Malassezia* species and antifungal susceptibility test could facilitate selection of appropriate antifungal agents.

Keywords: Pityriasis versicolor; *Malassezia*; *M. fur fur*

1. Background

Malassezia yeasts are lipophilic fungi which are regarded as normal flora of the skin, and are recovered in 75-98% of healthy individuals (1-3). *Malassezia* yeasts may also have a role in pityriasis versicolor (4), seborrhoeic dermatitis (5, 6), dandruff (6), seborrhoeic blepharitis (7), *Malassezia* folliculitis (8), confluent, reticulate papillomatosis (9) and uncommon systemic infections. In the last category, *Malassezia* species are associated with catheter acquired sepsis, fungaemia and pulmonary infection in neonates; these infections can sometimes be life threatening (10-14) and have been reported as significant causes of fungal infections in patients with cancer (15, 16) and HIV positive individual (17).

The difficulty in culturing *Malassezia* spp. *in vitro* has delayed the determination the taxonomic status of these yeasts. Nowadays, morphological characteristics and physiological features, the use of different Tween com-

pounds as well as molecular studies of *Malassezia* species have allowed their classification into 11 species; *M. furfur*, *M. sympodialis*, *M. slooffiae*, *M. obtusa*, *M. globosa*, *M. restricta*, *M. pachydermatis*, *M. yamatoensis*, *M. nana*, *M. japonica* and *M. dermatis* (18, 19). Since molecular methods are not cost effective and unavailable in clinical laboratories, thus the purpose of this study was to identify *Malassezia* species isolated from pityriasis versicolor patients in Sari, Iran, by conventional methods, based on morphological, physiological and biochemical techniques (20).

2. Materials and Methods

2.1. Sample Collection

This cross sectional study was conducted between October 2011 to April 2012 and a total of 134 patients clinically suspected of pityriasis versicolor, attending the Department of Mycology, Boali Sina Hospital and Referral Labo-

Implication for health policy/practice/research/medical education :

The results demonstrated a significant relationship between the frequency of *Malassezia* species and age, however, gender and anatomical site of lesions of pityriasis versicolor was not significant.

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ratory of the Mazandaran University of Medical Sciences were consecutively included. Individuals who received local or systemic antifungal for at least 5 days or corticosteroid agents for 3 weeks before the mycological examination were excluded from the study.

Skin scrapings were taken from the scaliest sites and obtained by the use of sterile blades. The diagnosis of pityriasis versicolor was established by the presence of both hyphae and spores in direct microscopy with KoH 20% and methylene blue staining. The assessed samples were plated onto Sabouraud's dextrose agar (SDA, Difco) and modified Leeming and Notman agar (1% w/v peptone, 1% w/v glucose, 0.2% w/v yeast extract, 0.8% desiccated ox bile, 0.1% v/v glycerol, 0.05% w/v glycerol monostearate, 0.5% v/v Tween 60, and 2% v/v oleic acid, 1% w/v agar in distilled water) supplemented with cyclohexamide (0.5%) and chloramphenicol (0.05%)(21), as fungal general medium and incubated at 32°C for two weeks in the dark. The study then proceeded as follows, to identify the *Malassezia* species. This research was approved by the ethics committee of Mazandaran University of Medical Science and written informed consent was obtained from the each participant prior to enrolment.

2.2. Microscopic and Physiological Characteristics

Among *Malassezia* species, only *M. pachydermatis* is capable of growing on Sabouraud's agar. However, more tests are essential for identification of the remaining *Malassezia* species such as Tween assimilation test, catalase reaction and splitting of esculin. After one week of incubation on modified Leeming and Notman agar at 32°C, the yeast cells were examined morphologically by gram and methylen blue staining. The catalase reaction was performed by the application of a drop of hydrogen peroxide (30% solution) onto a culture smear on a glass slide. Production of gas bubbles was considered as a positive reaction. *M. restricta* is the only lipid- dependent species lacking catalase (22, 23).

2.3. Splitting of Esculin

According to the method described by Mayser et al. (24), the b-glucosidase activity of different *Malassezia* species was assayed. Briefly, the loop of fresh yeast was deeply inoculated in the esculin agar (Merck, Germany) tube and incubated for 5 days at 32 °C. The splitting of esculin is indicated by darkening of the medium. This test was used to distinguish *M. furfur*, *M. slooffiae* and *M. sympodialis* from other *Malassezia* species.

2.4. Tween Assimilation Test

The ability to utilize individual Tween compounds (Tween 20, 40, 60 and 80) was tested according to Guillot

et al. (20). Briefly; the sterile Sabouraud's agar (16 ml) was melted and allowed to cool to approximately 50 °C. *Malassezia* yeast suspensions (about 10⁵ cfu/mL) were mixed with Sabouraud's agar, and the mixtures were plated. Five holes were made in the agar by the means of a 2 mm diameter punch and filled with 5 µl of Tween 20, 40, 60 and 80 (Merck, Germany). The plates were incubated for one week at 32° C. Utilization of Tween was assessed by the degree of growth and /or reaction (precipitation) of the lipophilic yeasts around individual wells.

2.5. Statistical Analysis

Data collected in checklists were analyses using SPSS version.16. The data were analyzed using the chi-squared test. A P value of < 0.05 was considered significant.

3. Results

Of the 134 skin scraping samples, 116 patients (86.5%) were confirmed with pityriasis versicolor, based on the presence of both hyphae and spores in direct microscopy with KoH 20% and methylene blue staining, however, the *Malassezia* spp. were isolated from 100/116 (86.2%) or 100/134 (74.6%) patients in the culture media, who were the final enrolled subjects. Table 1 shows that the distribution of patients clinically suspected of pityriasis versicolor based on age/ sex, indicating that there was no significant difference between age and gender of patients with pityriasis versicolor.

Table 1. Distribution of Patients Clinically Suspected of Pityriasis Versicolor Based on Age/Sex

Age, years	Male No. (%)	Female No. (%)	Total No. (%)
≤ 5	4 (3)	3 (2.2)	7 (5.2)
6 - 14	1 (9.7)	15 (11.2)	28 (20.9)
15 - 24	20 (14.9)	31 (23.1)	51 (8.1)
25 - 34	16 (11.9)	21 (15.7)	37 (27.6)
35 - 44	3 (2.2)	3 (2.2)	6 (4.5)
≥ 45	4 (3)	1 (.7)	5 (3.7)
Total	60 (44.8)	74 (55.2)	134 (100)

According to descending frequency for the presence of *Malassezia* species among patients, *M. globosa* was found in n = 54; 54% cases, *M. furfur* in n = 32; 32% cases, *M. sympodialis* in n = 6; 6% cases, *M. restricta* in n = 6; 6% cases and *M. slooffiae* in n = 2; 2% cases. In this survey *M. pachydermatis* and *M. obtusa* were not found in any of the cases. There was a significant association between age and *Malassezia* species frequency ($\chi^2 = 36.32$ df = 20, P = 0.014) (Table 2). There was no significant association between gender of patients and *Malassezia* species frequency ($\chi^2 = 9.24$ df = 4, P = 0.055) (Table 3). Table 4 has shown the frequency

of isolated *Malassezia* species based on anatomical locations. There was no significant association between ana-

tomical sites and *Malassezia* species frequency ($\chi^2 = 23.26$ df = 20, P = 0.28) (Table 4).

Table 2. *Malassezia* Species Frequency According to Age of Patients (P = 0.014)

<i>Malassezia</i> spp.	<i>M. globosa</i> %	<i>M. furfur</i> %	<i>M. sympodialis</i> %	<i>M. restricta</i> %	<i>M. slooffiae</i> %	Total, %
Age, y						
≤ 5	3	1	1	-	-	5
6 - 14	8	8	1	1	1	19
15 - 24	33	9	2	2	-	46
25 - 34	5	13	2	2	-	22
35 - 44	3	-	-	1	-	4
≥ 45	2	1	-	-	1	4
Total	54	32	6	6	2	100

Table 3. Frequency of *Malassezia* Species According to Gender (P = 0.055)

	<i>M. globosa</i> %	<i>M. furfur</i> %	<i>M. sympodialis</i> %	<i>M. restricta</i> %	<i>M. slooffiae</i> %	Total, %
Male	26	18	4	-	2	50
Female	28	14	2	6	-	50
Total	54	32	6	6	2	100

Table 4. Frequency of *Malassezia* Species According to the Lesion Site (P = 0.276)

	<i>M. globosa</i> %	<i>M. furfur</i> %	<i>M. sympodialis</i> %	<i>M. restricta</i> %	<i>M. slooffiae</i> %	Total, %
Chest	12	8	-	-	-	20
Back	11	5	3	5	1	25
Abdomen	14	9	1	-	1	25
Neck	9	3	1	-	-	13
Face	7	4	1	1	-	13
Arms	1	3	-	-	-	4
Total	54	32	6	6	2	100

4. Discussion

Although, *Malassezia* yeasts are part of the normal flora of the skin and are discovered in 75–98% of healthy hosts, their association with various skin disorders have been established; many studies have been conducted on the distribution of these yeasts (1-3). A great number of epidemiological studies using culture or molecular-based methods, e.g. PCR-RFLP or nested-PCR, have been performed in different geographical regions in order to investigate *Malassezia* species in patients with pityriasis versicolor (23, 25-27), including culture-based studies carried out in Spain (25, 28, 29), Japan (26), Bosnia and Herzegovina (30) Tunisia (27), Iran (31, 32), Turkey (33) and India (34).

By using morphological and physiological characteristics particularly in tropical areas, *M. globosa* either alone or associated with other *Malassezia* spp. (e.g. *M. sympodialis*, *M. slooffiae*) was the most commonly isolated *Malassezia* species. This ratio that indicated the predominance

of *M. globosa* was between 53.3% and 63.3% (25-28, 30-35). In contrast, a study carried out by Gupta et al. (36) indicated that *M. sympodialis* was the predominant pityriasis versicolor isolate (59.5%), followed by *M. globosa* (25.2%), *M. furfur* (10.8%), *M. slooffiae* (2.7%), *M. restricta* (0.9%) and *M. obtusa* (0.9%) in Canada. In the present study, the most predominant species observed was *M. globosa* (54%) followed by *M. furfur* (32%), *M. sympodialis* (6%), *M. restricta* (6%) and *M. slooffiae* (2%). Nevertheless, *M. pachydermatitis* and *M. obtusa* were not observed from pityriasis versicolor lesions.

Our findings for *M. globosa* were most compatible to that of other similar studies (25, 26, 28, 30-35). However, *M. furfur* and *M. slooffiae* seemed to occur more frequently than in other studies carried out in Canada (6) Spain (25, 28, 35), Japan (26) Iran (28,29), Tunisia (27) and Bosnia (25), in which a frequency between 0% and 25.3%, and 0% and 5.3% was reported, respectively. In this study, no significant differences were found in species isolated according to the clinical type or anatomical sites of lesions.

In contrast, in Bosnia, Prohic et al. (30) and in Spain, Erchiga et al. (25) reported that no significant differences were found in the isolated species according to the clinical sign or anatomical sites of lesions. In this study, the recovery rate of *Malassezia* spp. from pityriasis versicolor lesions was 74.6 % (100/134). In similar studies, this ratio tended to vary between 43.8% and 91.3% (17, 25-28, 30-34, 37-40).

It was noted that detection of different ratios of *Malassezia* species in patients with pityriasis versicolor in different parts of the world could be due to the use of different culture media (Leeming-Notman and modified Dixon agar) (25), climatic regions (tropical, subtropical, etc) (41), different sampling methods (swabbing/scraping) (25) and characteristics of patients (41). Although pityriasis versicolor has worldwide occurrence, its frequency is variable and depends on different climatic, occupational and socio-economic conditions (42, 43). In high temperate areas, the disorder is common in young adults aged 17-24 years. In tropical climates, pityriasis versicolor is more common in all age groups, but most cases occur in individuals aged 10-19 years (39).

In agreement with other investigations (43, 44), the highest prevalence of pityriasis versicolor in this study was observed for those aged between 15 to 24 years old, suggesting that the peak of the infection coincided with age periods when the sebum production is at its highest level. The role of gender in propensity to development of pityriasis versicolor is still unclear (29). To date the data revealed that pityriasis versicolor affected male individuals more than females (45, 46). However, several studies indicated that the incidence was higher in women (47, 48). In this survey we did not find significant association between gender of infected patients and the frequency of *Malassezia* species, this may be attributable to the extra attention of both sexes to beauty and skin hygiene (22).

Lesions mostly were found in the neck, face, back, arms, abdomen, and chest and in this survey, the most affected areas were found to be the chest, back and abdomen, which is concordance with the majority of studies worldwide (17, 49). The distribution of *Malassezia* species on the back and chest is proportional to the density and activity of pilosebaceous glands in these areas. However, there are a few reports indicated that pityriasis versicolor lesions can occur in unusual locations such as the nipple, genital areas and groin (49, 50).

5. Conclusions

According to our data, *M. globosa* was the main species causing pityriasis versicolor, as it was isolated from 54% of the lesions; followed by *M. furfur* (32% of lesions), while *M. pachydermatitis* and *M. obtuse* were not found in any of the cases and in 13.8% (16/116) of patients the culture was negative. The results demonstrated a significant relationship between the frequency of *Malassezia* species and age, however, gender and anatomical site of lesions of pityria-

sis versicolor was not significant. However, more studies with larger samples have to be performed to clarify the investigated subject. Since *Malassezia* species show different responses to antifungal therapy, thus correct identification of *Malassezia* species and antifungal susceptibility tests could facilitate selection of appropriate antifungal agents.

Acknowledgements

The authors are grateful to Dr. Nima Motamed for his help in part of the statistical analysis. We wish to thank the staff of Boali Sina Hospital and the Referral Laboratory of the Mazandaran University of Medical Sciences.

Authors' Contribution

None declared.

Financial Disclosure

None declared.

Funding/Support

The authors alone are responsible for the content and writing of the paper.

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