

Study of the Distribution of *Malassezia* Species in Patients with Pityriasis Versicolor in Kolar Region, Karnataka

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Abstract

Context: Pityriasis versicolor is a superficial, chronically recurring fungal infection caused by *Malassezia* species. Recently it has been revised taxonomically into 14 species, in that only 7 species have been well studied in relation to pityriasis versicolor. **Aims:** To identify *Malassezia* species isolated from patients with pityriasis versicolor and to find out any correlation between the species with clinical presentation of lesions. **Settings and Design:** Prospective study comprising of 100 clinically diagnosed cases of pityriasis versicolor attending Dermatology Outpatient Department over a period of 1 year. **Materials and Methods:** The clinical specimens were collected under aseptic precautions and subjected to culture on Sabouraud's Dextrose Agar overlaid with olive oil and modified Dixon agar. The isolates were identified by biochemical tests. **Statistical Analysis Used:** Statistical analysis was done using proportion, mean and chi-square test. **Results:** Of the 100 cases, 73% were males, 26% were females and predominant age group was 21-30 years. Out of 100 samples, 70 yielded growth. The most common isolate was *M. sympodialis* (50%), followed by *M. furfur* (32.86%), *M. globosa* (14.28%) and *M. slooffiae* (2.86%). Among 100 cases, 74% had hypopigmented and 26% had hyperpigmented lesions. *M. sympodialis* and *M. furfur* were predominantly isolated from hypopigmented lesions and *M. globosa* and *M. slooffiae* were found to be more common in hyperpigmented lesions. **Conclusions:** *M. sympodialis* was the most common isolate, followed by *M. furfur*, *M. globosa* and *M. slooffiae*. There was no significant difference in distribution of different species in patients with hypo or hyper pigmented lesions

Key Words: Hyperpigmentation, hypopigmentation, *Malassezia*, pityriasis versicolor

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What was known?

World wide it is believed that the causative agent of the common disease pityriasis versicolor is *M. globosa* then *M. furfur*.

Introduction

Pityriasis versicolor (PV) is a superficial, chronically recurring fungal infection of stratum corneum. The lesions are characterized by scaly, hypo or hyper pigmented irregular macules, most often seen on lipid-rich areas of the body, including upper part of trunk, neck, face and upper aspect of arms.^[1] Clinically, the disease is asymptomatic, usually patient seeks medical attention for cosmetic purposes.^[1] It has a worldwide distribution, though it is more frequent in tropical region (40%) due to relatively high temperature and humidity.^[2-4]

The etiological agents of this superficial surface infection belong to the genus *Malassezia*. The *Malassezia* is lipophilic dimorphic fungus, occurs as yeast form in

culture media and mycelia forms in the skin lesions.^[5] It has been recognized as a member of normal skin flora as well as organisms involved with superficial cutaneous infections.^[6] Under the influence of certain exogenous and endogenous factors, the commensal yeasts transform into filamentous pathogenic forms.^[7] The normal human skin flora consists predominantly the yeast phase where as mycelia phase predominates in skin lesions.^[8] Pathological specimens consists predominantly hyphae with clusters of spherical yeasts described as "spaghetti and meat ball" appearance.^[9]

Malassezia species have been associated with diverse dermatological lesions like pityriasis versicolor, seborrheic dermatitis, atopic dermatitis, pityrosporum folliculitis, psoriasis, onychomycosis and blepharitis. Though generally associated with very mild superficial infection, it is emerging as an opportunistic fungal pathogen in immunocompromised patients and as nosocomial pathogen in patients with central line vascular catheter, particularly in low birth weight neonates who receive lipid emulsions.^[10]

Currently genus *Malassezia* includes 14 species, namely *M. furfur*, *M. sympodialis*, *M. globosa*, *M. restricta*,

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M. slooffiae, *M. obtusa*, *M. dermatis*, *M. japonica* and *M. yamatoensis* associated with normal human flora and can cause skin lesions, *M. pachydermatis*, *M. nana*, *M. equina*, *M. caprae* and *M. cuniculi* are associated with animals.^[11,12]

Although there is evidence to suggest variation in geographical distribution of different species, not much data is available from our country. Therefore, this study was undertaken to study the distribution of different *Malassezia* species in Kolar region, Karnataka.

Materials and Methods

This study comprises of 100 clinically diagnosed cases of PV attending Dermatology Outpatient Department over a period of 1 year at R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka. A detailed history with reference to name, age, sex, place and clinical details like site of lesion and whether hypo or hyperpigmented were recorded. All patients clinically diagnosed as PV showing positive for presence of yeast in KOH (Potassium Hydroxide) mount were included in the study. Patients who have already received topical antifungal therapy within last 3 months and oral therapy within the last 6 months were excluded.

After taking informed consent skin scrapings from affected area are collected.^[10] Those samples that showed the typical "spaghetti and meat balls" appearance on 10% KOH examination were subjected to culture. The samples were inoculated into SDA (Sabourad's Dextrose Agar) slant overlaid with sterile olive oil, plain SDA and MDA (modified Dixon agar) plates and all the cultures were incubated at 32°C. The culture plates and slants were examined every day for growth and culture negatives were discarded only after 3 weeks of incubation.^[4,13]

The morphological features like budding pattern, shape of yeast cell were studied by observing Gram's stained smears on 5th day after colonies were noticed on culture media. Speciation of *Malassezia* was done using catalase test, esculin hydrolysis and assimilation of Tween 20, 40, 60 and 80.^[3,14-16] Statistical analysis was done with descriptive tools like proportion and mean. Proportional difference observed within groups was being compared using the Chi-square test.

Results

Of the 100 cases of PV, (73%) were males and (27%) were females, with a high male preponderance. The age of the

patients with PV ranged from 15 to 60 years. Majority of them were in the age group of 21-30 years (57%), followed by 31-40 years (27%), 11-20 years (10%), 41-50 years (4%) and 51-60 years (2%). Seventy-four (74%) had hypopigmented lesions and 26 (26%) had hyperpigmented lesions.

Distribution of PV lesions on different sites of the body showed that chest and trunk (30%) was the most common site involved, followed by back (27%), multiple sites (21%), these patients showed involvement of two or more sites, neck (12%), arm (8%) and axilla (2%).

Among 100 samples only 70 yielded growth on both SDA with olive oil and MDA. None of them showed any growth on plain SDA. The growth on SDA with olive oil appeared after 8 to 10 days of inoculation and on MDA within 5 days of inoculation.

Of the 70 culture positives, 35 (50%) yielded *M. sympodialis*, 23 (32.86%) *M. furfur*, 10 (14.28%) *M. globosa*, 2 (2.86%) *M. slooffiae*. The isolation of *M. sympodialis* is significantly ($P < 0.001$) higher than the isolation of other species.

Distribution of different species on different sites of the body shown in Table 1. *M. sympodialis* and *M. furfur* was isolated predominantly from lesions on the back, *M. globosa* from the chest and trunk, *M. slooffiae* was isolated from chest and trunk and also from lesions on neck and arm.

Of the 70 culture-positive patients, 52 (74.27%) had hypopigmented lesions and 18 (25.71%) had hyperpigmented lesions. Different species isolated with respect to type of lesion is shown Figure 1. *M. sympodialis* and *M. furfur* were predominantly isolated from hypopigmented lesions and *M. globosa* and *M. slooffiae* were found to be more common in hyperpigmented lesions.

Discussion

Yeasts of the genus *Malassezia* cause various skin lesions, among which PV is the most common and worldwide in occurrence.^[4] The genus *Malassezia* has undergone several taxonomic revisions. In the last reclassification, 14 distinct species are recognized, in that only 7 species have been well studied in relation to PV.^[11,12]

Table 1: Isolation of *Malassezia* species from different sites of the body

Species	Site n(%)						Total
	Chest and trunk	Back	Multiple sites	Neck	Arm	Axilla	
<i>M. Sympodialis</i>	6 (17.14)	14 (40)	8 (22.85)	2 (5.71)	5 (14.28)	-	35
<i>M. Furfur</i>	5 (21.74)	8 (34.78)	5 (21.74)	5 (21.74)	-	-	23
<i>M. Globosa</i>	6 (60)	1 (10)	1 (10)	1 (10)	-	1 (10)	10
<i>M. Slooffiae</i>	1 (50)	-	1 (50)	-	-	-	2

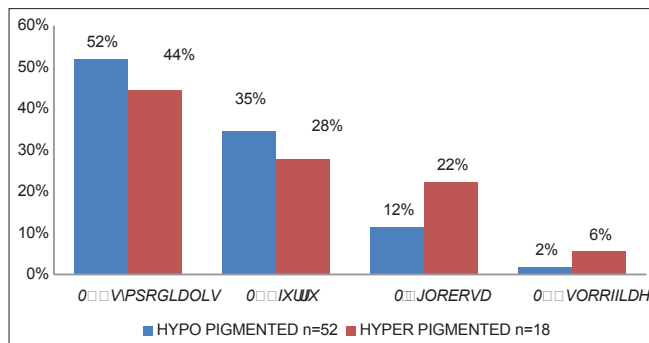


Figure 1: Distribution of *Malassezia* species in patients with hypo and hyper pigmented lesions

The incidence of PV was more common in males (73%) than in females (27%). Similar to our study Gosh *et al.*, Rao *et al.* and Krishnan *et al.* also found male predominance.^[1,2,17] The higher incidence of PV in males may be due to their outdoor activities.^[18]

Hypopigmented lesions were seen in 74% and hyperpigmented lesions in 26% of patients. Other studies from India, Gosh *et al.* (81% hypopigmented), Rao *et al.* (75% hypopigmented) and Krishnan *et al.* (84% hypopigmented) also showed predominance of hypopigmented lesions in PV.^[1,2,17] In general, PV is thought to have a tendency to be hypopigmented in dark skinned individuals and hyper pigmented in fair skinned individuals. However, Aljabre *et al.* studied pigmentary changes in patients and concluded that PV is not necessarily hypopigmented in dark-skinned individuals and there is no correlation between pigment variation and the type of skin, sex and age of patient and site of lesion.^[19]

The occurrence of PV was more common in adult age group than in the pediatric age group. In adults it was found to be more common in 21-30 year age group (57%). This association was found to be statistically significant with $P < 0.001$. A similar finding was also reported by Rao *et al.* (21-30yr), Krishnan *et al.* (15-29yr), Dutta *et al.* (11-30yr) and Tarazooie *et al.* (20-30yr).^[2,17,20,21] In contrast, el-Hefnawi *et al.* reported highest prevalence in the third and fourth decade of life.^[22] In the study by Akapata *et al.* it was found that majority of cases occurred during adolescence.^[23] This was believed to be due to hormonal changes and increase in sebum secretion during this period.^[23]

The most common site of involvement was chest and trunk, followed by back, multiple sites involving two or more sites, neck, arm and axilla. Similar findings are also noted by Dutta *et al.*, Rao *et al.* and Tarazooie *et al.* with chest and trunk, back and neck as most commonly involved sites. Possibly because of the distribution of sebaceous glands is higher in these areas.^[2,20,21]

The isolation rate of *Malassezia* species in patients with pityriasis versicolor in the current study is 70%,

which is comparable to that of Kindo *et al.* (68.57%) from south India. In contrast to our study it is higher in Chaudhary *et al.* (96.66%) from central India and Shokoshi *et al.* (88.4%) from Iran.^[13,15,24] The growth on ordinary media like SDA with olive oil appeared after 8 to 10 days of inoculation and growth on special media like MDA appeared early, within 5 days of inoculation. Preparation of these special media is difficult and they are expensive when compared to SDA. Use of simple media like SDA overlaid with olive oil for isolation of lipid-dependent *Malassezia* species is cost effective and can be used in any routine diagnostic laboratory.^[3]

The distribution of *Malassezia* species varies with different geographical locations. In the present study, the most common *Malassezia* species isolated was *M. sympodialis*. Kindo *et al.* from South India also reports *M. sympodialis* as the predominant species.^[15] Similar reports also available from other parts of world such as Canada and Indonesia, where they found *M. sympodialis* as predominant species in PV.^[3,8,25] In contrast, Chaudhary *et al.* from Central India and Dutta *et al.*, Kaur *et al.* from North India reported *M. globosa* as the most common isolate.^[4,13,20] *M. furfur* was the second most frequent species isolated from PV in this study, which is comparable to Dutta *et al.*^[20] Our study showed *M. globosa* as third common isolate, which is contrary to other studies from India which report it as the most common species causing PV.^[13,20] In our study *M. slooffiae* was isolated from two (2.86%) cases of PV which is almost similar to Tarazooie *et al.* (4%) from Iran; however, none of the other Indian studies have isolated *M. slooffiae* from PV.^[21] *M. globosa* and *M. restricta* are predominant species isolated in non culture based epidemiological studies.^[26]

Distribution of *Malassezia* species in lesions from different sites of the body showed that *M. sympodialis* and *M. furfur* were more common in lesions on the back, *M. globosa* from lesions on the chest and trunk, *M. slooffiae* was isolated from chest and trunk and also from lesions on neck and arms.

In our study, *M. sympodialis* and *M. furfur* were predominantly isolated from hypopigmented lesions, whereas *M. globosa* and *M. slooffiae* were found to be more common in hyperpigmented lesions. But this distribution is statistically not significant, may be because of lower sample size.

Use of simple media like SDA overlaid with olive oil for isolation of lipid dependent *Malassezia* species is cost effective and can be used in any routine diagnostic laboratory.^[3] Speciation of *Malassezia* is based on simple biochemical tests such as catalase reaction, esculin hydrolysis and Tween assimilation. These tests are simple to perform and cost effective and can be used in routine diagnostic laboratory. Molecular

methods such as RFLP (restriction fragment length polymorphism), nested-PCR (polymerase chain reaction) or PCR-REA (polymerase chain reaction-restriction endonuclease analysis) are being developed to hasten the identification process and resolve the difficulty in interpretation of some physiological patterns. Although molecular techniques are most sensitive methods for identification, they are expensive and technically demanding.^[13]

What is new?

This study shows that *M. sympodialis* was the predominant isolate followed by *M. furfur*, *M. globosa* and *M. slooffiae*.

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