

# Isolation and Speciation of *Malassezia* in Patients Clinically Suspected of Pityriasis Versicolor

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## Abstract

**Introduction:** *Malassezia* species are lipophilic yeasts recognized as commensals of skin that may be pathogenic under certain conditions. The most common described human infection due to a member of the genus is pityriasis versicolor (PV), a chronic, superficial disease of the stratum corneum layer of the epidermis containing the typical hyphal elements and yeast cells of *Malassezia furfur*.

**Objectives:** Identify and speciate *Malassezia* in patients clinically suspected of having PV.

**Methodology:** The study was conducted at the Department of Dermatology and Microbiology at Kempegowda Institute of Medical Sciences, Bengaluru, from July 2012 to June 2014. All clinically suspected cases coming to the Department of Microbiology were subjected to mycological work-up. Skin scrapings were microscopically examined with 10% potassium hydroxide (KOH) for budding yeast cells and fungal filaments and inoculated for culture using modified Dixon's media and Sabouraud's dextrose agar with and without olive oil at various temperatures. The growth in the culture tubes and plates were further speciated using biochemical tests.

**Results:** Total cases in the period of 2 years were 130. Male to female ratio is 1.6:1. The infection was common in the age group of 20-30 years. The common clinical presentation was hypopigmented lesion over the shoulder, arm, chest, and back. Out of the 130 suspected cases, KOH was positive in 120 and culture was positive in 130. Wood's lamp examination was positive in 100 cases, negative in 5 and it was not done in 25 cases. Wood's lamp positive cases were also positive for KOH and culture.

**Conclusion:** The most common species isolated was *M. furfur* followed by *Malassezia slooffiae*, *Malassezia pachydermatis*, *Malassezia globosa*, *Malassezia obtusa*, and *Malassezia restricta*. A prospective study was aimed to determine the incidence of *Malassezia* in patients clinically suspected of having PV and to speciate the identified isolates.

**Key words:** *Malassezia*, Pityriasis versicolor, Potassium hydroxide

## INTRODUCTION

*Malassezia* is a lipophilic yeast found in areas rich in sebaceous glands of the human skin and other warm-blooded animals.<sup>1</sup> It is a part of normal flora of the human skin. In up to 90%

of adults, *Malassezia* is commonly found in seborrheic areas and becomes pathogenic in warm and humid environment.<sup>2</sup> The most common disease caused by *Malassezia* is pityriasis versicolor (PV).<sup>2</sup> *Malassezia* genus includes 12 species comprising of *Malassezia furfur*, *Malassezia pachydermatis*, *Malassezia sympodialis*, *Malassezia globosa*, *Malassezia obtusa*, *Malassezia restricta*, *Malassezia slooffiae*, *Malassezia dermatis*, *Malassezia japonica*, *Malassezia nana*, *Malassezia yamatoensis*, and *Malassezia equi*. The first seven species have been studied in relation to PV.<sup>2</sup> PV is a mild, chronic, usually asymptomatic superficial fungal infection<sup>3</sup> that appears as round-to-oval lesions commonly found on the trunk and upper aspects of the arms. These lesions vary in color and can be hypo- or

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hyperpigmented<sup>4</sup> or erythematous scaly skin lesions of the stratum corneum.<sup>1</sup> These yeasts are also associated with clinical infections such as folliculitis, seborrheic dermatitis, atopic dermatitis, confluent and reticulate papillomatosis, and systemic infections.<sup>5,6</sup> The disease PV was described by Eichstedt in 1846, though he was unable to isolate the organism. Robin in 1853 isolated fungal elements from these lesions and named the organism as *Microsporum furfur*, since it was identical to the dermatophyte *Microsporum audouinii*. The disease was later renamed as tinea versicolor. These yeast cells were isolated from human dandruff scales by Malassez in 1874, and Baillon included this group of yeasts under the genus *Malassezia*. Sabouraud (1904) considered this organism as a cause of dandruff and gave it a new name, *Pityrosporum malassez*. In 1984, *Malassezia* gained priority over *Pityrosporum* and was accepted as the generic name for the fungus.<sup>7</sup> *Malassezia* has also been shown to be associated with invasive infections in immunocompromised patients, especially those receiving intravenous lipids or with central venous catheters.<sup>1</sup> It is believed that endogenous factors such as increased sweating, administration of corticosteroids, malnutrition and increased plasma cortisol level, and exogenous factors like high temperature and humidity mediate the development of PV.<sup>1</sup> In spite of treatment, the recurrence rate of *Malassezia* is about 60% in the 1<sup>st</sup> year and 80% in the 2<sup>nd</sup> year.<sup>1</sup> If the disease is left untreated, it causes cosmetic problems of neck, face, trunk, etc.<sup>1</sup> In order to prevent morbidity, recurrence and invasive infections, early laboratory diagnosis of the condition is required.<sup>1</sup> Hence, a prospective study was aimed to determine the incidence of *Malassezia* in patients clinically suspected of having PV and speciate the identified isolates. Numerous studies have shown the relationship between these species and their role as causative agents in triggering of diseases.<sup>4</sup> Present study was undertaken to isolate and speciate *Malassezia* in patients clinically suspected of PV.

## OBJECTIVES

- Identify and speciate *Malassezia* in patients clinically suspected of having PV.

## METHODOLOGY

The present study was conducted in the Department of Dermatology and Microbiology at Kempegowda Institute of Medical Sciences, Bengaluru, for 2 years, from July 2012 to June 2014. Consent was taken from all clinically suspected cases and after a detailed clinical history they were subjected to mycological work up.

Lesions of patients clinically suspected of having PV were examined under Wood's lamp for the presence of golden

yellow fluorescence in the Department of Dermatology. Those lesions that showed golden yellow fluorescence were wood's lamp positive and the others that did not show fluorescence were Wood's lamp negative.

### Specimen Collection

After selection of an appropriate site, the affected area was cleaned aseptically with 70% ethyl alcohol and allowed to dry. Skin scrapings were done from the edges of the lesion using sterile scalpel or with the edge of a clean glass slide. The scrapings were subjected to both microscopy and directly inoculated onto the culture.

### Microscopic Examination

Direct microscopic examination was undertaken by a wet mount preparation with 10% potassium hydroxide (KOH). It was placed in the incubator at 37°C for 15 min and then examined to detect the presence of hyphae and budding yeast cells, which generally exhibit the characteristic appearance of "spaghetti and meatballs (Figure 1)."<sup>8,9</sup>

### Culture Study

After the area is cleaned aseptically with 70% ethyl alcohol, the fine scales were collected directly onto modified Dixon's media containing chloramphenicol and Sabouraud's dextrose agar (SDA). SDA used were SDA with actidione and without olive oil at room temperature and at 37°C, SDA with actidione with olive oil at room temperature and at 37°C. The inoculated slants and plates were observed every day for the suspected growth of *Malassezia* for 7-21 days before negative results were noted. After the growth is seen, Gram's-staining was done to see for the Gram-positive budding yeast cells (Figures 2 and 3).

### Biochemical Test

The gross morphology of suspected colonies of *Malassezia* was noted, and speciation was performed using the different biochemical tests such as the catalase test using 30% hydrogen peroxide, bile esculin splitting test, and cremaphore test using 10% castor oil and tween 20, 40, 80 utilization test was done by standard procedure and based on the results, speciation was done for *Malassezia*.

### Catalase Test

Production of gas bubbles on adding a drop of 30% hydrogen peroxide indicated a positive reaction (Figure 4).

### Bile Esculin Splitting Test

The colonies are streaked on bile esculin agar and development of black color indicated a positive test (Figure 5).

### Tween (20, 40, 80) Utilization Tests

A lawn culture of the organism is prepared on SDA plate, and three wells are made into which tween 20, 40, 80 are

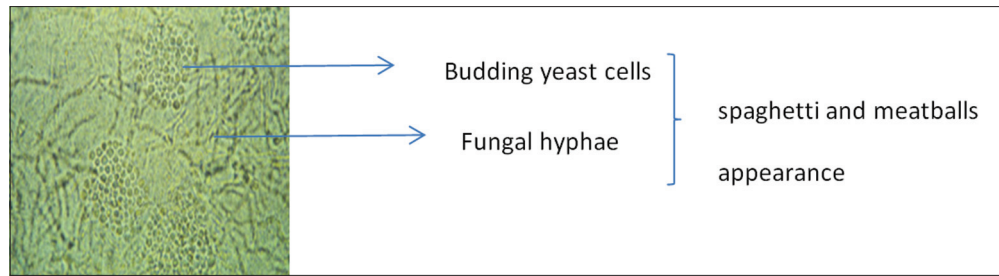


Figure 1: Potassium hydroxide preparation



Figure 2: Growth on modified Dixon's media

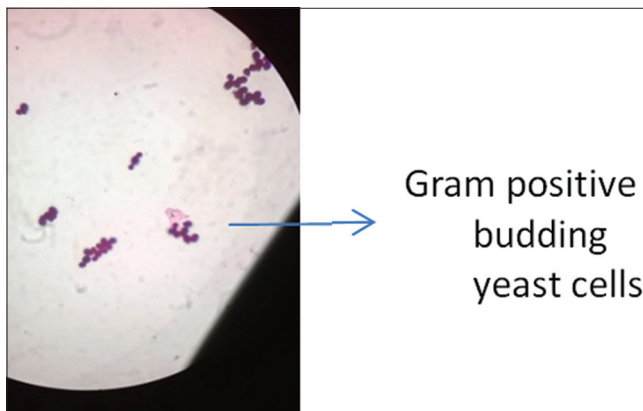


Figure 3: Gram's-staining

inoculated. Utilization of tweens was assessed by the degree of growth and/or reaction (precipitation) of the lipophilic yeasts around individual wells (Figure 6).

## RESULTS

Total suspected cases of PV during the 2 years period was 130. The male to female ratio for the present study was 1.6:1. Out of the 130 suspected cases, 80 were males (62%) and 50 were females (38%) who presented with the clinical features of PV (Figure 7).

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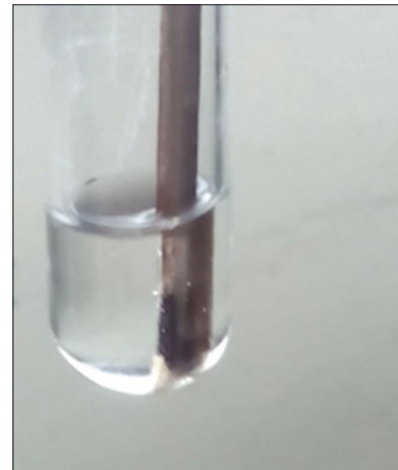


Figure 4: Catalase test



Figure 5: Bile esculin test

of PV. The highest prevalence of PV was observed in 20-30 years old age group with 47%, and the least affected were <10 years and >50 years (Figure 8).

Among the 130 cases, 90 cases presented with the hypopigmented lesion and 40 cases were hyperpigmented (Figure 9).

### Wood's Lamp Examination

Out of the 130 cases, Wood's lamp was positive in 100 cases and negative in 5. It was not done in 25 cases (Figure 10).

Out of the 130 cases, KOH and culture was positive in 120 cases, 10 cases that were KOH negative was culture positive (Table 1).

Wood's lamp examination was done for 105 cases.

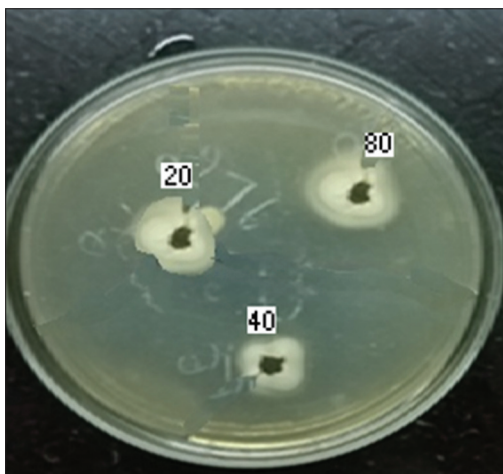


Figure 6: Tween (20, 40, 80) utilization tests

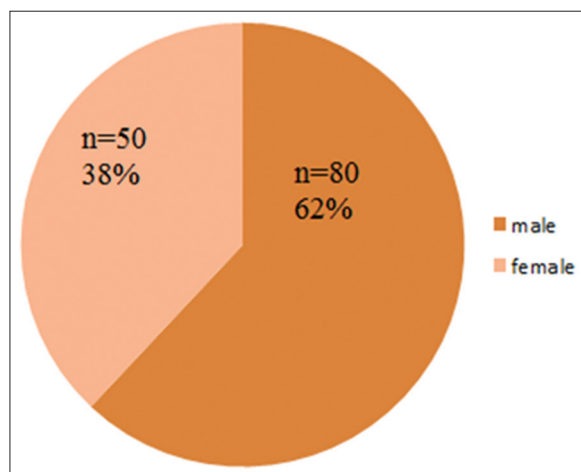


Figure 7: Sex distribution of clinically suspected cases

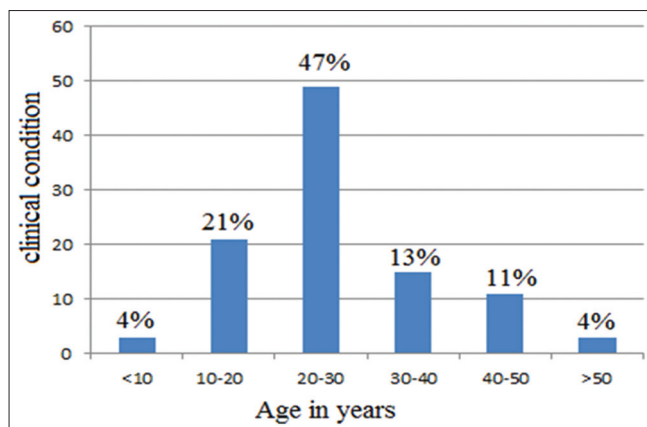


Figure 8: Distribution of pityriasis versicolor among different age groups

Out of the 130 cases, the Wood's lamp examination showed yellow fluorescence in 100 cases and was negative in 5. All 105 cases were both KOH and culture positive (Table 2).

Wood's lamp examination was not done in 25 patients. Of the 25 cases where Wood's lamp examination was not done, KOH was positive in 15 and negative in 10 but, all 25 cases were culture positive (Table 3).

Table 4 differentiates the various species based on their biochemical reactions. All *Malassezia* species are lipid-dependent except *M. pachydermatis*. The species are

Table 1: Comparison of KOH and culture results

KOH	Culture	Total
Positive	Positive	120
Negative	Positive	10

KOH: Potassium hydroxide

Table 2: Comparison of Wood's lamp result, KOH, and culture

Tests done	Positive	Negative
Wood's lamp examination	100	5
KOH	105	0
Culture	105	0

KOH: Potassium hydroxide

Table 3: Comparison of Wood's lamp result, KOH, and culture

Test	Positive	Negative
KOH	15	10
Culture	25	0

KOH: Potassium hydroxide

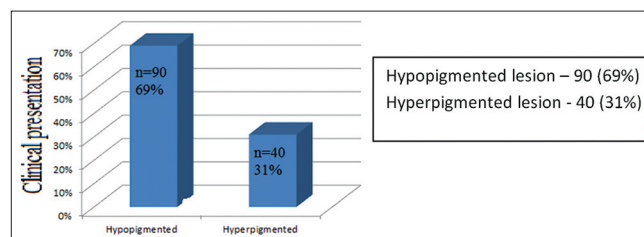


Figure 9: Distribution of clinical presentation of the patients

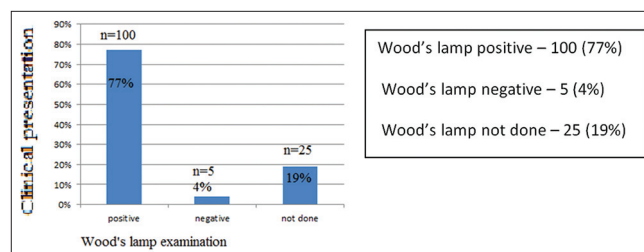


Figure 10: Distribution of Wood's lamp examination results



**Table 4: Distribution of species based on their different biochemical reactions**

Species	Lipid-dependence	Growth at 37°C	Catalase reaction	Esculin splitting	Cremaphore (10%)	Tween 20	Tween 40	Tween 80
<i>M. furfur</i>	+	+	+	-	V	+	+	+
<i>M. pachydermatis</i>	-	+	V	V	V	+	+	+
<i>M. sympodialis</i>	+	+	+	+	-	-	+	+
<i>M. globosa</i>	+	-	+	-	-	-	-	-
<i>M. obtusa</i>	+	-	+	+	-	-	-	-
<i>M. restricta</i>	+	-	-	-	-	-	-	-
<i>M. slooffiae</i>	+	+	+	-	-	+	+	-

*M. furfur*: *Malassezia furfur*, *M. pachydermatis*: *Malassezia pachydermatis*, *M. sympodialis*: *Malassezia sympodialis*, *M. globosa*: *Malassezia globosa*, *M. obtusa*: *Malassezia obtusa*, *M. restricta*: *Malassezia restricta*, *M. slooffiae*: *Malassezia slooffiae*

differentiated based on their growth, catalase reaction, esculin splitting, growth with cremaphore, and tweens at various concentrations.

Figure 11 shows the different species isolated - *M. furfur* is the most common isolate (52%) followed by *M. slooffiae*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*.

Table 5 shows the distribution of various species of *Malassezia* based on the body sites. *M. furfur* has been isolated from shoulder, arm, chest, neck, and back. *M. slooffiae* and *M. pachydermatis* have been isolated from the chest, *M. sympodialis* and *M. globosa* has been isolated from back, *M. obtusa* from shoulder and arm and *M. restricta* from chest lesion.

## DISCUSSION

Association of *Malassezia* with various skin disorders such as PV, seborrheic dermatitis, and *Malassezia* folliculitis,<sup>10</sup> has been well-known. The frequency of recovery from *Malassezia* depends on various factors such as age, sex, body sites, and differences in techniques of identification.

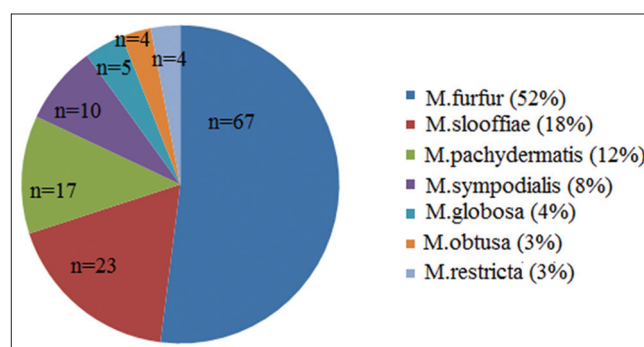
The total cases that came to the department of microbiology during the 2 years period were 130. 80 males (58%) and 50 females (42%) were suspected of PV. Male to female ratio was 1.4:1. Many studies by Shah *et al.*,<sup>1</sup> Shoeib *et al.*,<sup>2</sup> Ghosh *et al.*,<sup>3</sup> and Kaur *et al.*,<sup>5</sup> have shown the same pattern of male predominance. The male preponderance is because they are more involved with outdoor activities, which place them at high risk of exposure to factors such as high temperatures and humidity.<sup>1</sup>

The highest prevalence of PV was observed in 20-30 years old age group, and the least affected were <10 years and >50 years. Studies by Shah *et al.*,<sup>1</sup> Shoeib *et al.*,<sup>2</sup> Tarazooie *et al.*,<sup>4</sup> and Kaur *et al.*,<sup>5</sup> has correlated with our findings. This indicated that the disease generally appears in the late teens, with a peak in the 20 years, when the sebum production is at the highest level and rarely found in

**Table 5: Distribution of *Malassezia* species based on the body sites in PV**

Species	Face	Neck	Chest	Back	Shoulder and arm	Abdomen
<i>M. furfur</i> (67)	5	10	15	10	25	2
<i>M. slooffiae</i> (23)	3	10	10	-	-	-
<i>M. pachydermatis</i> (17)	3	-	10	2	2	-
<i>M. sympodialis</i> (10)	-	-	2	4	4	-
<i>M. globosa</i> (5)	1	-	-	3	-	1
<i>M. obtusa</i> (4)	-	-	-	-	2	2
<i>M. restricta</i> (4)	-	1	2	-	1	-

PV: Pityriasis versicolor, *M. furfur*: *Malassezia furfur*, *M. slooffiae*: *Malassezia slooffiae*, *M. pachydermatis*: *Malassezia pachydermatis*, *M. sympodialis*: *Malassezia sympodialis*, *M. globosa*: *Malassezia globosa*, *M. obtusa*: *Malassezia obtusa*, *M. restricta*: *Malassezia restricta*

**Figure 11: Different species of *Malassezia* isolated**

elderly individuals. The most affected areas of the body were the trunk, shoulder, and arm, and least affected site were abdomen and face. These findings correlated with other studies of Shah *et al.*,<sup>1</sup> Shoeib *et al.*,<sup>2</sup> and Kaur *et al.*,<sup>5</sup> In the present study, 90 cases showed the hypopigmented lesion and 40 cases were hyperpigmented. Shah *et al.*, Shoeib *et al.*, Kaur *et al.*, and Bitra *et al.*, have reported hypopigmented lesions as a common clinical presentation. Two cases presented with both hypopigmented and hyperpigmented lesions. Two different species were isolated in the above cases - *M. globosa* and *M. restricta* in one of the cases and *M. globosa* and *M. obtusa* in another case. Such finding has not been reported from any other studies. The hypopigmentation induced by this fungus can be explained on the basis of production of

dicarboxylic acids, a main component of which is azelaic acid. This acid acts through competitive inhibition of 3,4-dihydroxyphenylalanine tyrosinase and perhaps has a direct cytotoxic effect on hyperactive melanocytes. The pathogenesis of hyperpigmentation is also not fully understood, but it may be due to increased thickness of the keratin layer and more pronounced inflammatory cell infiltrate in these individuals that act as a stimulus for the melanocytes.<sup>9</sup>

Out of the 130 cases the Wood's lamp examination showed yellow fluorescence in 100 (77%) cases and all wood's lamp positive was culture positive. Five cases were wood's lamp negative but culture positive. In 25 cases, Wood's lamp was not done but *Malassezia* species were isolated in culture.

10% KOH was performed on all 130 cases, out of which, 120 samples were KOH positive, and all 130 samples were culture positive. 10 samples which were KOH negative, showed culture positive. In our study, *M. furfur* was the most common species that was isolated (52%), followed by *M. slooffiae* (18%), *M. pachydermatis* (12%), and *M. sympodialis* (8%), *M. globosa* (4%), *M. restricta* (3%), and *M. obtusa* (3%). In studies by Shah *et al.*,<sup>1</sup> *M. globosa* (50%), Shoeib *et al.*, *M. sympodialis* (54%), Ghosh *et al.*,<sup>3</sup> and *M. globosa* (53%), and in the study by Kaur *et al.*,<sup>5</sup> *M. furfur* (52%) was the common isolate.

## CONCLUSION

*Malassezia* species can now be added to a growing list of normal skin flora organisms of low virulence that may cause mild recurrent skin infections and serious systemic infection in the susceptible host. Although, *Malassezia* species are considered as normal microflora of the human skin, these lipophilic yeasts are associated with many skin disorders in particular PV.<sup>5</sup> The identification

of *Malassezia* yeast to species level is of importance to determine which species are implicated in skin disease and whether there is variation in the distribution of the yeast with clinical data, body site, and origin of the population, etc.<sup>5</sup> Biomolecular techniques are considered as fast and more accurate methods to identify *Malassezia* species.<sup>6</sup> However, simple methods based on morphology and physiology evaluation, such as the ones employed in this study, are more accessible to every laboratory.<sup>6</sup> It would help to prevent recurrences, systemic complications and any cosmetological problems especially in patients of younger age group.

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