

## Original Research Article

# DISTRIBUTION OF MALASSEZIA SPECIES IN PATIENTS WITH PITYRIASIS VERSICOLOR AT A TERTIARY CARE HOSPITAL

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## ABSTRACT

**Background:** Pityriasis versicolor (PV) is a superficial fungal infection caused by *Malassezia* species, part of the normal skin flora. Species-level identification and antifungal susceptibility profiling are essential due to variable clinical presentations and emerging resistance.

**Materials and Methods:** A cross-sectional study was conducted over one year (July 2017–June 2018) in Coimbatore Medical College Hospital. Skin scrapings from 102 clinically diagnosed PV cases were examined microscopically and cultured on Modified Dixon agar. Isolates were speciated, and antifungal susceptibility testing was performed using the broth microdilution method (CLSI M27-A3).

**Results:** Most PV cases occurred in males (65.7%) aged 15–25 years (44.1%). The chest (39.2%) was the most common site of lesion. KOH mount was positive in 88% of cases, and culture positivity was 73.5%. *M. globosa* (60%) was the predominant species, followed by *M. sympodialis* (28%) and *M. furfur* (12%). Increased sweating was a significant risk factor ( $p < 0.05$ ). Itraconazole showed 100% sensitivity across all isolates, while resistance to fluconazole was noted, particularly in *M. globosa*.

**Conclusion:** PV is more common in young males, with *M. globosa* being the predominant species. Increased fluconazole resistance highlights the need for regional surveillance and susceptibility testing to guide effective antifungal therapy.

**Keywords:** Pityriasis versicolor, *Malassezia*, antifungal susceptibility, ketoconazole, fluconazole, itraconazole, Modified Dixon agar.

## INTRODUCTION

Fungal infections of the skin are a significant global health issue and are broadly classified into cutaneous, subcutaneous, systemic, and opportunistic mycoses.<sup>[1]</sup> Among these, superficial mycoses are the most common, involving the stratum corneum with minimal tissue reaction.<sup>[2]</sup> Pityriasis versicolor (PV), also known as tinea versicolor, is a prevalent superficial fungal infection caused by lipophilic yeasts of the genus *Malassezia*, which are part of the normal skin flora, especially in sebaceous gland-rich areas.<sup>[3,4]</sup> *Malassezia* species thrive in sebum-rich environments and are found in warm-blooded vertebrates.<sup>[5]</sup> PV appears as chronic,

recurrent scaly macules that induce dyspigmentation—hypopigmented lesions in darker skin and hyperpigmented spots in fair-skinned people—and typically affects the chest and back.<sup>[6]</sup> The illness is particularly frequent in tropical regions such as India, with rates of up to 40-50%, but less common in colder climes.<sup>[7]</sup> Cultural opinions toward PV differ; for example, in some parts of Sri Lanka, the lesions are regarded beauty marks known as gomora, or tears of liquid gold.<sup>[8,9]</sup> *Malassezia* inhibits melanin formation by secreting dicarboxylic acids such as azelaic acid, which causes pigmentation changes in PV.<sup>[10]</sup>

PV affects both sexes equally, with a small masculine advantage due to outdoor activity and

sweating.<sup>[11]</sup> It is most common among teens and young adults aged 21 to 30.<sup>[12]</sup> The illness is not communicable or caused by poor hygiene, although it is linked to malnutrition, heat, humidity, excessive sweating, and systemic diseases such as diabetes, Cushing's syndrome, immunosuppression, pregnancy, and corticosteroid use.<sup>[13,14]</sup> Aside from PV, *Malassezia* species cause a variety of dermatological diseases, including seborrheic dermatitis, atopic dermatitis, and pityrosporum folliculitis.<sup>[15]</sup> *Malassezia* bacteria can cause systemic infections in immunocompromised newborns receiving complete parenteral feeding.<sup>[16]</sup> Taxonomically, *Malassezia* yeasts belong to Basidiomycetes.<sup>[17]</sup> Since their initial classification in 1996, about 14 species have been identified, including *M. furfur*, *M. globosa*, *M. restricta*, and others.<sup>[18]</sup> These species vary in their distribution and pathogenicity, influenced by geographic and host factors.<sup>[19]</sup> Diagnosis of PV relies on microscopic examination of skin scrapings treated with 10% KOH, revealing the characteristic "spaghetti and meatballs" fungal elements.<sup>[20]</sup> Wood's lamp examination may aid diagnosis through yellow-green fluorescence.<sup>[21]</sup> Culturing requires lipid-enriched media like Modified Dixon agar, as most species are lipophilic, except *M. pachydermatis*, which can grow on standard media without lipids.<sup>[22]</sup> Species differentiation employs biochemical tests such as catalase, urease, and Tween assimilation.<sup>[23]</sup> PV has a significant recurrence rate—up to 60% in the first year and 80% in the second year after therapy.<sup>[3,24]</sup> Untreated infections can lead to invasive fungal illness, especially in immunocompromised hosts.<sup>[25]</sup> Early and precise diagnosis is thus critical to lowering morbidity and recurrence.<sup>[26]</sup> The purpose of this study is to investigate the incidence and distribution of *Malassezia* species in patients with pityriasis versicolor at a tertiary care hospital, which will provide insight into species prevalence and aid in improved clinical management.

## MATERIALS AND METHODS

**Study Design and Setting:** This cross-sectional study was conducted at the Department of Microbiology, Coimbatore Medical College Hospital, in collaboration with the Department of Dermatology, over one year (July 2017 to June 2018).

**Study Population:** The study included 102 clinically diagnosed patients with pityriasis versicolor attending the Dermatology outpatient department (OPD). Both male and female patients of all ages presenting with hypopigmented or hyperpigmented macular lesions were enrolled.

**Ethical Considerations:** Approval was obtained from the Institutional Ethical Committee prior to the study. Written informed consent was obtained from all participants, who were interviewed using a

structured questionnaire to collect demographic and clinical data, including history of illness, family history, and risk factors such as diabetes, steroid use, and immunocompromised status.

**Sample Collection:** Skin scrapings were collected from the active edges of lesions after cleansing with 70% isopropyl alcohol. The samples were processed according to standard mycological procedures.

**Microscopic Examination:** Samples were examined using 10% potassium hydroxide (KOH) wet mounts to detect yeast cells and fungal hyphae, showing the characteristic 'spaghetti and meatball' appearance of *Malassezia*. Parker Quink's stain was also used for enhanced visualization of fungal elements.

**Culture:** Samples were cultured on Sabouraud's dextrose agar (SDA) with olive oil overlay and Modified Dixon's agar, incubated at 32°C for 7–14 days. Colony morphology was observed daily.

**Identification of *Malassezia* Species:** Species identification was done by examining colony morphology, Gram staining, and biochemical tests, including:

Lipid dependence test

Urease test

Catalase test

Growth at 41°C

Bile esculin splitting test

Tween assimilation test

These tests helped differentiate *Malassezia* species based on growth characteristics and enzymatic activity.

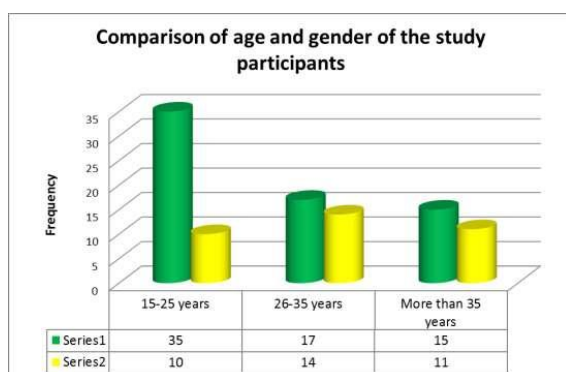
**Antifungal Susceptibility Testing:** Minimum inhibitory concentrations (MICs) for ketoconazole, fluconazole, and itraconazole were determined using the broth microdilution method according to CLSI guidelines. Stock solutions were prepared in DMSO (for water-insoluble drugs) or distilled water (for water-soluble drugs). Serial dilutions were made in RPMI-1640 medium supplemented with lipids. The inoculum was standardized to  $0.5\text{--}2.5 \times 10^3$  CFU/ml. Microtiter plates were incubated at 32°C for 48–72 hours. Growth and drug controls were included.<sup>[27]</sup>

**Interpretation:** MIC was defined as the lowest drug concentration that inhibited visible growth. Since no standard breakpoints exist for *Malassezia*, criteria established for *Candida krusei* were used. For fluconazole, MIC  $\leq 2$  µg/ml was considered susceptible; for ketoconazole, MIC  $\leq 1$  µg/ml was susceptible.

**Statistical Analysis:** Data were entered into Excel and analyzed using SPSS version 24. Categorical variables were expressed as frequencies and percentages, and continuous variables as mean  $\pm$  standard deviation. Associations were tested using chi-square and independent t-tests, with  $p < 0.05$  considered statistically significant.

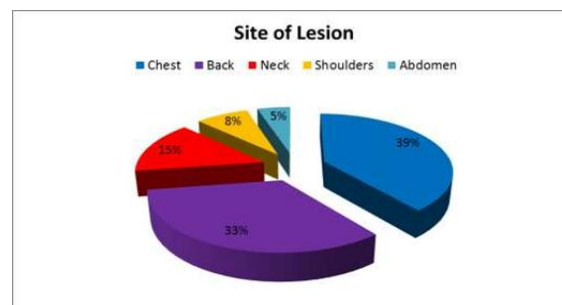
## RESULTS

This study was conducted over a period of one year, from July 2017 to June 2018, at the Departments of Microbiology and Dermatology, Coimbatore Medical College Hospital, Coimbatore. A total of 102 patients attending the Dermatology OPD, clinically diagnosed with pityriasis versicolor (PV) and confirmed by the characteristic spaghetti and meatball appearance on 10% potassium hydroxide (KOH) mount, were included. Out of 102 patients diagnosed with pityriasis versicolor, 67 (65.7%) were males and 35 (34.3%) were females. The largest number of cases (44.1%) occurred in the 15-25 years age group, followed by 30.3% in the 26-35 years group, and 25.4% in those older than 35 years. Notably, males in the 15-25 years age group were significantly more affected compared to females, with a p-value of 0.02, indicating a statistically significant association. The median age of occurrence was 24 years [Figure 1].



**Figure 1: Distribution of Age and Gender in Pityriasis Versicolor Cases (n=102)**

The chest was the most commonly affected site in PV patients, observed in 39.21% of cases, followed by the back in 33.3% of cases. Other affected sites included the neck (14.8%), shoulders (7.8%), and abdomen (4.9%). This distribution suggests that the upper trunk is the primary region involved in PV [Figure 2].



**Figure 2: Distribution of Site of Lesion in Pityriasis Versicolor**

Out of 102 patients, 90 (88%) tested positive for PV by KOH wet mount. Among these, 75 cases (73.5%) were confirmed by both KOH and fungal culture, whereas 15 cases (14.7%) were KOH positive but culture negative. Twelve patients (11.8%) were negative by both diagnostic methods, indicating that KOH mount is a highly sensitive diagnostic tool for PV, with culture providing confirmatory evidence [Table 1].

**Table 1: Association Between 10% KOH and Culture Positivity in PV Cases**

10% KOH Wet Mount	Culture Positive	Culture Negative	Total
Positive	75 (73.5%)	15 (14.7%)	90 (88%)
Negative	0	12 (11.8%)	12 (11.8%)
Total	75 (73.5%)	27 (26.5%)	102

Among the 75 culture-positive isolates, *Malassezia globosa* was the most frequently isolated species, accounting for 60% of cases. *M. sympodialis* was the second most common at 28%, followed by *M.*

*furfur* at 12% [Table 2]. This suggests *M. globosa* is the predominant species involved in PV infections in this population.

**Table 2: Distribution of Malassezia Species Among Culture Positive Cases**

Fungal Species	Number of Isolates	Percentage (%)
<i>M. globosa</i>	45	60
<i>M. sympodialis</i>	21	28
<i>M. furfur</i>	9	12
Total	75	100

Increased sweating was the most significant risk factor associated with culture-positive PV cases, observed in 62.7% of patients and showing strong statistical significance ( $p = 0.0001$ ). Diabetes mellitus (30.7%) and steroid usage (4%) were also

noted among the patients but were not statistically significant risk factors ( $p > 0.05$ ) [Table 3]. This indicates that hyperhidrosis may play a critical role in PV susceptibility.

**Table 3: Association of Risk Factors with Culture Positivity for Malassezia spp. on Modified Dixon Agar**

Risk Factor	Culture Positive No.	Percentage (%)	Chi-square Value	P value
Increased Sweating	47	62.7	15.1	0.0001*
Diabetes Mellitus	23	30.7	1.47	0.225
Steroid Usage	3	4	0.005	0.946
No Risk Factors	2	2.6	0.004	0.986

*M. globosa* was the most commonly isolated species in both hypopigmented (59.4%) and hyperpigmented (66.7%) lesions. *M. sympodialis* and *M. furfur* were less frequently isolated, and no significant association was found between the type

of lesion and the species of *Malassezia* ( $p = 0.639$ ) [Table 4]. This suggests that the type of pigmentation in PV lesions is not influenced by the specific *Malassezia* species.

**Table 4: Association Between Type of Lesion and Malassezia Species in PV Patients**

Species	Hypopigmentation No. (%)	Hyperpigmentation No. (%)	Chi-square Value	P value
<i>M. globosa</i>	41 (59.4%)	4 (66.7%)	0.897	0.639
<i>M. sympodialis</i>	19 (27.5%)	2 (33.3%)		
<i>M. furfur</i>	9 (13.0%)	0 (0.0%)		

Minimum inhibitory concentration (MIC) testing showed that *M. globosa* isolates had ketoconazole MICs ranging from 0.03 to 4 µg/ml, fluconazole MICs from 0.06 to 8 µg/ml, and itraconazole MICs from 0.03 to 0.125 µg/ml. *M. sympodialis* and *M.*

*furfur* showed similar MIC ranges but with slightly lower upper limits [Table 5]. These findings indicate a broad susceptibility of *Malassezia* species to these antifungal agents within the tested concentration ranges.

**Table 5: MIC Ranges of Antifungal Agents Tested Against Malassezia Species**

Species	Ketoconazole MIC (µg/ml)	Fluconazole MIC (µg/ml)	Itraconazole MIC (µg/ml)
<i>M. globosa</i>	0.03 - 4	0.06 - 8	0.03 - 0.125
<i>M. sympodialis</i>	0.06 - 0.25	0.06 - 0.5	0.03 - 0.25
<i>M. furfur</i>	0.125 - 0.5	0.03 - 0.5	0.06 - 0.125

Among the isolates, 86.6% of *M. globosa* were sensitive to ketoconazole, with 13.3% showing resistance. For fluconazole, 77.7% of *M. globosa* isolates were sensitive, while 22.2% were resistant. All *M. globosa* isolates were sensitive to itraconazole. Similarly, all *M. furfur* isolates showed 100% sensitivity to ketoconazole, fluconazole, and

itraconazole. *M. sympodialis* isolates were 100% sensitive to ketoconazole, but only 66.6% were sensitive to fluconazole [Table 6]. No resistance to itraconazole was observed in any species, suggesting itraconazole as the most effective antifungal agent tested.

**Table 6: MIC Interpretation of Ketoconazole, Fluconazole & Itraconazole Against Different Malassezia Species**

Species	Ketoconazole Sensitive ( $\leq 1\mu\text{g/ml}$ )	Ketoconazole Resistant ( $>1\mu\text{g/ml}$ )	Fluconazole Sensitive ( $\leq 2\mu\text{g/ml}$ )	Fluconazole Resistant ( $>2\mu\text{g/ml}$ )	Itraconazole Sensitive ( $\leq 1\mu\text{g/ml}$ )	Itraconazole Resistant ( $>1\mu\text{g/ml}$ )
<i>M. globosa</i> (45)	39 (86.6%)	6 (13.3%)	35 (77.7%)	10 (22.2%)	45 (100%)	0
<i>M. sympodialis</i> (21)	21 (100%)	0	14 (66.6%)	7 (33.3%)	21 (100%)	0
<i>M. furfur</i> (9)	9 (100%)	0	9 (100%)	0	9 (100%)	0

## DISCUSSION

This cross-sectional study was conducted over a period of one year, from July 2017 to June 2018, in the Departments of Microbiology and Dermatology at Coimbatore Medical College Hospital. A total of 102 patients attending the Dermatology outpatient department (OPD) with hypopigmented or hyperpigmented macular rashes were clinically diagnosed with pityriasis versicolor (PV), and their diagnosis was confirmed using 10% KOH wet mount.

**Age and Gender Distribution:** The majority of cases (44.1%) were observed in the 15–25 years age

group, followed by the 26–35 years group (30.3%). This pattern aligns with findings from Biswas Sampurna et al., where 40.2% of cases were in the 15–25 years group,<sup>[28]</sup> and Archana BR et al., who reported 57% of cases in the same age group.<sup>[29]</sup> Other studies, including those by Sharma et al., Shah et al., and Ghosh et al., also reported a higher prevalence among adolescents and young adults.<sup>[30–32]</sup> The increased incidence in this age group may be attributed to greater sebaceous gland activity, which provides the lipid-rich environment necessary for *Malassezia* growth.<sup>[33]</sup> PV remains uncommon in the elderly and in children. In the present study, males (65.7%) were more commonly

affected than females (34.3%).<sup>[34]</sup> This male predominance is supported by studies from Kumar S et al. (62% males),<sup>[35]</sup> Chaudhary et al. (80% males),<sup>[36]</sup> and others such as Shah et al., Ghosh et al., Rao et al., and Krishnan et al.<sup>[31,32,37,38]</sup> A statistically significant association was observed between age and gender ( $p < 0.05$ ), similar to the study by Sharma et al.<sup>[39]</sup> The higher prevalence in males could be related to increased outdoor activity, greater exposure to heat and sweat, and the resultant favorable environment for fungal colonization.<sup>[40]</sup>

**Site of Lesion:** The chest (39.2%) was the most commonly affected site, followed by the back (33.3%), neck (14.8%), shoulders (7.8%), and abdomen (4.9%).<sup>[41]</sup> These findings correspond with studies by Ghosh et al., where the chest and back were the predominant sites.<sup>[32]</sup> Conversely, other studies such as those by Rezvan et al. and Shah et al. identified the neck as the most frequently affected area.<sup>[31,42]</sup> The distribution of lesions typically reflects areas with high densities of sebaceous glands and exposure to humid environments, both of which facilitate fungal growth.<sup>[43]</sup>

**KOH and Culture Positivity:** In this study, 88.2% of cases were positive by 10% KOH mount. Similar positivity rates were observed in studies by Shah et al. (82%),<sup>[31]</sup> Kaur et al. (89%),<sup>[44]</sup> Chaudhary et al. (90%),<sup>[36]</sup> and Kumar et al. (92.2%).<sup>[35]</sup> KOH mount remains a reliable, simple, and cost-effective diagnostic method, providing characteristic "spaghetti and meatball" appearance, even in culture-negative cases.<sup>[45]</sup> The culture positivity rate was 73.5%, which aligns with the findings of Archana BR et al. (70%),<sup>[46]</sup> and Jasim M Karhhot et al. (79%).<sup>[47]</sup> Of the 90 KOH-positive cases, 75 (73.5%) were culture-positive, while 15 were culture-negative. Similar findings were reported by Kindo et al. (68.6%),<sup>[48]</sup> and Tarazooie et al. (79%).<sup>[49]</sup> Differences in culture positivity may result from variations in sampling technique, prior antifungal use, or the lipid-dependence of *Malassezia* and the use of modified culture media.<sup>[50]</sup>

**Species Distribution:** The predominant species isolated was *M. globosa* (60%), followed by *M. sympodialis* (28%) and *M. furfur* (12%). These results are consistent with studies by Nakabayashi et al.,<sup>[51]</sup> and Crespo-Erchiga et al., who reported *M. globosa* as the leading isolate.<sup>[52]</sup> Other studies by Dutta et al.,<sup>[53]</sup> and Tarazooie et al. also support this finding.<sup>[49]</sup> However, studies by Makimura et al. and Gupta et al. suggest geographical variation, where *M. furfur* and *M. sympodialis* may predominate in certain temperate regions.<sup>[54,55]</sup>

**Risk Factors:** Among culture-positive patients, the most common risk factor was increased sweating (62.7%), followed by diabetes mellitus (30.7%) and steroid usage (4%).<sup>[56]</sup> Increased sweating was significantly associated with PV ( $p < 0.05$ ). This finding supports studies by Tarazooie et al. and Faergemann, which identified hyperhidrosis as a key predisposing factor.<sup>[49,58]</sup> Although diabetes and

steroid use were also present, their associations were not statistically significant, consistent with studies by Ghosh et al., Krishnan et al., and Gatha Rao et al.<sup>[32,37,38]</sup>

### Type of Lesion and Species Correlation:

Hypopigmented lesions were more common than hyperpigmented lesions, in line with studies by Chaudhary et al., Shah et al., and Krishnan et al.<sup>[31,36, 37]</sup> PV tends to present as hypopigmented lesions in darker skin types and hyperpigmented in fair skin. However, Aljabre et al. noted no clear correlation between pigmentation and skin type.<sup>[58]</sup> In this study, *M. globosa* was the predominant species in both hypo- and hyperpigmented lesions, though the association between species and lesion type was not statistically significant ( $p > 0.05$ ). This contrasts with the findings of Archana BR et al., who reported that *M. sympodialis* and *M. furfur* were more often found in hypopigmented lesions.<sup>[29]</sup>

### Antifungal Susceptibility and MIC

Minimum Inhibitory Concentration (MIC) testing showed the following ranges: ketoconazole (0.03–4 µg/ml), fluconazole (0.03–8 µg/ml), and itraconazole (0.03–0.25 µg/ml). *M. globosa* exhibited broader MIC ranges and higher resistance compared to *M. sympodialis* and *M. furfur*. All isolates of *M. furfur* and *M. sympodialis* were 100% sensitive to ketoconazole and itraconazole, while resistance was observed mainly in *M. globosa* to fluconazole and ketoconazole. These findings are consistent with studies by Gupta et al., Velagraki et al., and Florencia et al., which noted higher MIC values for fluconazole.<sup>[59,60,61]</sup> Itraconazole showed the most consistent susceptibility across all species tested, suggesting it may be the most effective systemic agent for PV.<sup>[62]</sup> Among species, *M. sympodialis* demonstrated the highest overall susceptibility, followed by *M. furfur*, with *M. globosa* showing the greatest resistance.<sup>[63]</sup> Although MIC testing is valuable for identifying antifungal resistance, standardized interpretive breakpoints for *Malassezia* species are lacking. Further research is needed to correlate in vitro MIC values with clinical outcomes to guide treatment strategies effectively.

## CONCLUSION

*Malassezia* species, though part of the normal skin flora, can cause superficial infections like pityriasis versicolor (PV), especially in young adults. In this study, PV was most common in males aged 15–25 years. *M. globosa* was the predominant species in both hypopigmented and hyperpigmented lesions, with a higher isolation rate from hyperpigmented lesions. Direct microscopy and culture on Modified Dixon agar are essential for diagnosis and species identification. Antifungal susceptibility testing showed increased resistance to fluconazole, while ketoconazole and itraconazole were more effective. As standard antifungal breakpoints for *Malassezia* are not yet established, local surveillance

of species distribution and resistance patterns is crucial. This helps guide effective treatment and prevent recurrence or chronic skin issues.

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