

## Isolation and Speciation of Malassezia in Patients with Clinically Suspected Pityriasis Versicolor at a Tertiary Care Hospital

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### Original Research Article

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**Abstract:** Malassezia is opportunistic lipophilic yeast found in areas rich in sebaceous glands of human beings and animals. Pityriasis versicolor (PV) is the most common skin disease caused by genus Malassezia, characterized by patchy discoloration of skin ranging from hypo to hyper pigmentation or erythematous skin lesions of stratum corneum. It has also been shown to be associated with deep invasive infections in immunocompromised patients, especially those receiving intravenous lipids or with central venous catheters. Recurrence rate of Malassezia in spite of treatment is high, about 60% in first year and about 80% in second year. Thus, in order to prevent morbidity, recurrence and invasive infections, early laboratory diagnosis and treatment of the condition is required. Aim of the present study is to identify and speciate Malassezia isolates from clinically suspected cases of Pityriasis Versicolor and to know the local distribution of various species. 60 skin scrapings collected from clinical Pityriasis cases were evaluated by microscopy using 10% KOH mount and culture on Sabouraud's Dextrose Agar (SDA) with and without overlaid sterile olive oil and modified Dixon Agar. The isolates were speciated using biochemical tests. Of the 60 skin scrapings 48 (80%) were KOH positive and 38 (63.3%) were culture positive. Of the 12 KOH negative 4 were culture positive. Most frequent isolate was M.globosa (50%) followed by M.symptodialis (23.8 %), M.furfur (15.8%), M.obtusa (5.2%), M.pachydermatis (5.2%). The most common affected sites were neck 51% followed by back 48% and in 28% cases both back and chest were affected. The study of distribution of various species of Malassezia and their relationship with dermatologic disorders helps in improving the management and preventing the recurrence.

**Keywords:** Pityriasis versicolor, Malassezia, lipophilic yeast, 10% KOH, SDA.

## INTRODUCTION

The members of genus Malassezia are considered as resident flora of human beings and animals and produce clinical disease under favourable conditions such as exposure to hot humid climate. This fungus grows readily on skin surface rich in sebum, consisting of sterol ester, Squalene, Tri-glycerides and fatty acids. Yeast changes to mycelial phase, which is responsible for the clinical disease of Pityriasis Versicolor (P V). However, various species are responsible for superficial and systemic infections, P V being the most common presenting ailment [1].

PV is asymptomatic, mild, chronic, recurrent superficial fungal infection of stratum corneum characterised by patchy discoloration of skin ranging

from hyper to hypo pigmentation. Most of the patients are healthy individuals and seek medical advice only on cosmetic grounds, multiple well defined, non-inflammatory macular lesions with fine scaling which are discrete and vary in appearance. i.e hypo or hyper pigmented. Occasionally lesions take papular appearance. In cases where hair follicles are involved, it would result in folliculitis. Seborrheic Dermatitis is seen over areas rich in sebaceous glands i.e scalp, face, chest, back and flexural areas [1].

Other clinical conditions caused by Malassezia are Atopic Dermatitis, Systemic infections like septicaemia, bronchopneumonia reported in premature neonates, immune compromised individuals and patients on deep vein catheters receiving parenteral

nutrition [1]. Diagnosis of PV is based on typical clinical picture and laboratory findings.

Seven species were proposed previously and currently the genus *Malassezia* comprises 11 species based on molecular, morphological and biochemical profiles [2]. All the species are lipophilic, except *M.pachydermatis*. Other species include *M.furfur*, *M.symphodialis*, *M.globosa*, *M.obtusa*, *M.restricta*, *M.slooffiae*, *M.dermatis*, *M.japonica*, *M.nana*, & *M.yamotensis* [3].

The present cross sectional study was aimed to identify and speciate *Malassezia* isolates from clinically suspected cases of PV and to identify the prevalence of various species.

### MATERIALS AND METHODS

The study included 60 skin scrapings from Dermatology patients clinically diagnosed as Pityriasis versicolor (Fig 1) during August to December 2015. Under aseptic conditions, skin scrapings were collected in Whatman filter paper with sterile scalpel blade [3] after taking informed consent. Scrapings were subjected to 10% KOH mount and microscopic examination

under 40 xs to detect hyphae and yeast forms, for characteristic appearance of “Spaghetti and Meatballs” (Fig 2). Skin scrapings were inoculated on two slants of SDA with chloramphenicol & cycloheximide in which one was layered with sterile olive oil (Fig 3) and Modified Dixon agar [4] (Fig 4). Both were incubated at 32°C for 2 weeks and observed for growth after 48 hours up to 2 weeks. Usually growth occurs within 3- 4 days [2].

Gram’s stain of the culture isolate revealed small budding yeast cells lying singly and in loose clusters. The isolates were speciated using the characteristics such as lipid dependence, catalase reaction, growth at >37°C, esculin splitting, utilization of tween 20, 40 and 80 according to standard guidelines. (Table 1) [1].

*Malassezia* isolate was suspended in 2 ml distilled water, mixed with sterile SDA at 50°C and plated. After solidification 3 wells were made & filled with 5 uL each of Tween 20, 40, 80 and incubated at 32°C for 2 weeks [2]. Degree of growth = utilization of Tween (Fig – 5 & 6).



Fig-1: Hypopigmented patches

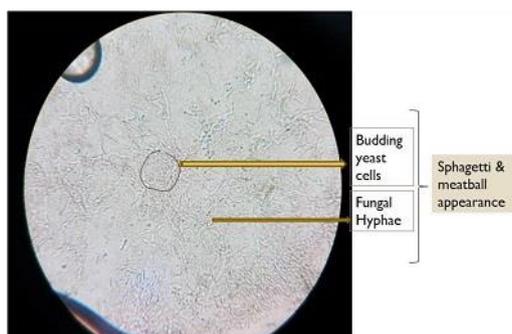


Fig-2: Spaghetti & meatball appearance in 10% KOH mount under 40 x objectives



Fig-3: Growth on SDA with and without olive oil overlay



Fig-4: Growth on modified Dixon agar

Table-1: Speciation characteristics of Malassezia

Malassezia species	Lipid dependence	Growth at 37°C	Catalase Reaction	Esculin splitting	Tween 20 (high Con)	Tween 40 (0.1-10%)	Tween 80 (low conc)
M. furfur	+	+	+	-	+	+	+
M.pachydermatis	-	+	V	V	+	+	+
M.sympodialis	+	+	+	+	-	+	+
M.globosa	+	-	+	-	-	-	-
M.obtusa	+	-	+	+	-	-	-
M.restricta	+	-	-	-	-	-	-
M.sloofiae	+	+	+	-	+	+	-



Fig-5: Negative Tween utilization test



Fig-6: Positive Tween utilisation

## RESULTS

Among the total 60 samples 48 (80%) were from males, 40 (66.6%) were in the age group between 20 - 30yrs. Students and labourers were 25 (41.6%) each. The most common affected body sites were neck 51% followed by back 48% and in 28% cases both back

and chest was affected. Of the 60 skin scrapings 48 (80%) were 10% KOH mount positive and 38 (63.3%) were culture positive. Of the 12 KOH mount negative 4 were culture positive. Results of culture on mDixon agar and SDA overlaid with sterile olive oil yielded similar results. By biochemical identification most

frequent isolate was *M. globosa* 19 (50%) followed by *M. sympodialis* 9(23.8%), *M. furfur* 6 (15.8%), *M. obtusa* and *M. pachydermatis* were 2 (5.2%) each.

## DISCUSSION

The frequency of recovery of *Malassezia* from PV depends on age, sex, profession and body site. Of the total 60 clinically suspected pityriasis Versicolor 40 (66.6 %) were in the age group 20 to 30yrs. and was similar to findings of Shah A *et al.* [6] because sebum production is more in this age group. Majority were males (80%) because they are more involved with outdoor activities, exposure to humidity and high temperatures.

Of the 60 cases of PV 58 (96.6%) were hypopigmented in the present study. The production of azelaic acid by the fungus acts through competitive inhibition of DOPA tyrosinase and perhaps has direct cytotoxic effect on hyperactive melanocytes leads to hypopigmentation [1]. Most common affected site in our study was neck (51%) followed by back (48%), both back and chest (28%) and similar to the findings of M Kaur *et al.* [3]. Majority were students (41%) followed by labourers (41%), businessmen and housewives (9%) each.

Most frequent isolate in our study was *M. globosa* (50%) and correlates with findings of Chaudhary *et al.* [7] as it is more pathogenic because of its enzymatic activity. However AJ Kindo *et al.* [2] in their research reported *M. sympodialis* was the predominant isolate followed by *M. globosa*.

## CONCLUSION

Isolation and identification of various species of *Malassezia* helps in understanding their relationship to recurrent dermatologic infections and helps in improving the management and preventing the recurrence which is a cosmetological problem.

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