



Original Research Article

## Isolation and Characterization of Different *Malassezia* Species from Pityriasis Versicolor Patients in Tertiary Care Hospital in Assam

Saveta Choraria Agarwal<sup>1</sup>, Ajanta Sharma<sup>2</sup>, Naba Kumar Hazarika<sup>3</sup>, Debeeka Hazarika<sup>4</sup>

<sup>1</sup>Consultant Microbiologist, Ramakrishna Mission Seva Prathisthan, Kolkata.

<sup>2</sup>Professor, Department of Microbiology, Assam Medical College and Hospital.

<sup>3</sup>Professor & HOD, Department of Microbiology, Guwahati Medical College and Hospital.

<sup>4</sup>Professor & HOD, Department of Dermatology, Venereology and Leprology, Guwahati Medical College and Hospital, Assam, India.

Corresponding Author: Saveta Choraria Agarwal

Received: 02/07/2015

Revised: 20/07/2015

Accepted: 27/07/2015

### ABSTRACT

**Background:** Since 1996, the taxonomic revision on the genus *Malassezia* in different studies have shown varying geographical distribution of different *Malassezia* species in pityriasis versicolor cases.

**Objective:** Isolation and characterisation of different *Malassezia* species in pityriasis versicolor patients.

**Materials and Methods:** Total 100 patients with pityriasis versicolor lesions were included. Samples were taken from the lesions for direct microscopy by KOH and parker's stain and were cultured in Sabouraud's dextrose agar (containing chloramphenicol and cycloheximide with olive oil overlay) and modified Dixon agar. Isolates were identified by standard morphological, biochemical and physiological characteristics.

**Results:** 10% KOH smear examination (with parker staining) showed 99% of positive result with characteristic sphagetti and meat ball appearance amongst 100 samples and 62% of the samples were culture positive. Out of these 50% of isolates were *M.globosa*, 20% were *M.furfur*, 17.7% were *M.obtusa* and 4.8% were found to be *M.slooffiae*. *M.sympodialis* was present in only 3.3% of the cases and 1.6% isolates were *M.restricta* and *M.dermatis* respectively.

**Conclusion:** The commonest species isolated was *M.globosa* followed by *M.furfur*, *M.obtusa*, *M.slooffiae*, *M.sympodialis*, *M.restricta* and *M.dermatis*.

**Keywords:** Pityriasis versicolor, *Malassezia*, KOH mount, Skin scrapings, Modified Dixon Agar

### INTRODUCTION

The last few decades have witnessed a persistent rise in global incidence and prevalence of mycotic diseases affecting humans. The genus *Malassezia* comprising of obligatory and non-obligatory lipophilic yeasts are also a part of the microbial community on normal skin which can

become pathogenic and can cause chronic and superficial skin infection under certain conditions and pityriasis versicolor is amongst one of them.<sup>[1]</sup>

Currently, *Malassezia* species has been expanded to include 14 species comprising *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M.*

*restricta*, *M. slooffiae*, *M. dermatis*, *M. japonica*, *M. nana*, *M. yamatoensis* and three not formally recognized species *M. caprae* & *M. equine* and recently *M. cuniculi*. The first seven species have been well studied in relation to pityriasis versicolor the most common disease caused by *Malassezia*. [2,3,4]

In the last 10 years studies have shown interesting geographical variation in the prevalence of different *Malassezia* species in pityriasis versicolor. Moreover, *Malassezia* species are more frequent in tropical and subtropical areas with high temperature and humidity. [2] Therefore, this study has been undertaken to establish whether there is any association between the various species of *Malassezia* and pityriasis versicolor as there are only few reports from India and none from North-east region.

## MATERIALS AND METHODS

Total of 100 samples were collected from the patients clinically diagnosed with pityriasis versicolor attending outpatients Department of Dermatology, Venereology and Leprology and processed in the Department of Microbiology, Gauhati Medical College and Hospital. Skin Scrapings were taken from the erythematous, peripheral, actively growing margins of the lesions. The study then processed as follows to identify the different species of *Malassezia*.

**Direct microscopy:** Morphological characteristics of yeasts were identified in 10% KOH mount with glycerol and also with Parker's ink (Parker Stain) and by fluorescent staining with calcofluor white.

**Culture:** Each specimen were inoculated in Sabourauds Dextrose Agar (SDA) with chloramphenicol and cycloheximide in doubles, one with olive oil overlay and another without it and were also inoculated in Modified Dixon agar for better isolation and visualization. Cultures were incubated at

31<sup>0</sup>C and colony characteristics were studied within one week extending up to 3 weeks if no growth noted.

### Tests for identification and differentiation of species:

#### 1. Gram Stain:

Any growth if noted was stained and examined for the presence of yeast cells which mostly appeared gram variable in case of *Malassezia*.

#### 2. Phenotypes characterization:

**a. Catalase Reaction:** Production of gas bubbles on addition of a drop of H<sub>2</sub>O<sub>2</sub> (3% solution) was considered as a positive reaction.

**b. Tween Assimilation Test:** Utilization of tween was assessed by the degree of growth of the lipophilic yeasts around the wells of the plates filled with 5 $\mu$ l of Tween 20, 40, 60 and 80 respectively which were incubated at 31<sup>0</sup>C for one week.

**c. Assimilation of Cremophor EL:** The plates were incubated at 31<sup>0</sup>C for 10 days and assessed for growth around the individual wells after 2,4,6,8 & 10 days.

**d. Splitting of Esculin:** The splitting of Esculin is revealed by darkening of the Esculin agar media incubated at 31<sup>0</sup>C for 5 days.

**e. Ability to Grow At 40<sup>0</sup>C:** was performed with the isolates.

**f. Glycine Assimilation Test:** Positive test is indicated by the growth of the isolates along the streaked done on the Modified Dixon agar with glycine media incubated at 37<sup>0</sup>C for 3 days.

## RESULTS

Of the 100 patients (73% male, 29% female) 41% were in the age group 20-30 years with lesions present commonly on the back (71%) followed by neck and upper

limbs with frequency of 57% each. Trunk (49%) and face (41%) were also involved. In 86 % the lesions were hypopigmented followed by either mixed hypo and hyperpigmented (7%) or only hyperpigmented (6%) lesions

63% of the patients presented in 1year of duration of this disease and 48% had mild to moderate itching. Wood’s light examination was positive in 65% cases. 1% KOH mount smear was negative while in 99% of the cases characteristic clusters of yeast cells with hyphae were observed (sphagetti and meat ball appearance). Of the 99 cases growth was obtained from 62% cases. 50% isolates were *M.globosa* followed by 21% for *M.furfur*, 17.7% for *M.obtusa*, 4.8% for *M.slooffiae*, 3% for *M.symphodialis*, 1.6% for *M. restricta* and *M.dermatis* respectively as shown in table1 and figure1

Most common predisposing factor found to be associated with pityriasis versicolor cases and Malassezia culture positive cases was seasonal variation in the frequency 80% & 83.4% respectively with

most of the cases occurring in summer season. 71% of the cases showed the association with hyperhydrosis. Genetic predisposition was present in 26% of the cases.

*M.globosa* had stable spherical cells on gram stain with buds formed on the narrow base; *M.symphodialis* had small ovoid cells with sympodial budding which is a characteristic feature. *M.furfur* showed characteristic glycine assimilation. *M.obtusa* showed cylindrical cells. The catalase reaction was positive for all except *M.restricta* as it consistently lack catalase. The tween assimilation test allowed the differentiation of most Malassezia species.

Table 1: Showing the Distribution of Malassezia Species in Pityriasis Versicolor Cases

MALASSEZIA SPECIES	PITYRIASIS VERSICOLOR CASES	
	NO.	PERCENTAGE
<i>M. dermatis</i>	1	1.6
<i>M. furfur</i>	13	21
<i>M. globosa</i>	31	50
<i>M. obtusa</i>	11	17.7
<i>M. restricta</i>	1	1.6
<i>M. symphodialis</i>	2	3.3
<i>M. slooffiae</i>	3	4.8
TOTAL	62	100

Table 2: Showing the Rate of Isolation of Three Commonest Malassezia Species in Different Studies and Present Study

REF NO.	PLACE	AUTHOR NAME	RATE OF ISOLATION OF <i>M.globosa</i> (%)	RATE OF ISOLATION OF <i>M.furfur</i> (%)	RATE OF ISOLATION OF <i>M.symphodialis</i> (%)
5	SPAIN	CRESPO-ERCHIGA <i>et al.</i> (1999)	55	0	11
6	JAPAN	NAKABAYASHI <i>et al.</i> (2000)	55	5	9
7	SPAIN	ASPIROZ <i>et al.</i> (2002)	58.2	0	9.8
8	INDIA	DUTTA <i>et al.</i> (2002)	54	30	0
9	IRAN	TARAZOOIE <i>et al.</i> (2004)	53.3	37.7	9
10	INDIA	KINDO <i>et al.</i> (2004)	39.6	0	58.3
2	INDIA	CHAUDHARY <i>et al.</i> (2010)	58	7	17
		PRESENT STUDY	50	21	3

## DISSCUSSION

The present study was done at a tertiary care hospital and it revealed a number of facts, many of which were in conformity with the previous studies. Out of the 62% culture positive samples, a single species was isolated in all cases. The most frequently isolated species was *M.globosa* (50%) followed by *M.furfur* (21%). The results of present study are most comparable

to the similar studies of Crespo-erchiga *et al.* (1999), Nakabayashi *et al.* (2000) and Aspiroz *et al.* (2002) in which *M.globosa* was isolated at the frequencies of 55%, 55% and 58.2% respectively. [5,6,7]

The study done in India by Dutta *et al.* (2002) found *M.globosa* (54%) to be the commonest species followed by *M.furfur* (30%) and these results were similar to the result observed in the present study. A

similar study from Iran by Tarazoe et al. (2004) found *M.globosa* (53.3%) and *M.furfur* (37.7%) as the commonest isolates. [8,9]

The highest prevalence of *M.globosa* in this study may be due to the fact that *M.globosa* pathogenicity might be related to its high lipolytic activity as suggested by Aspiroz et al. (2002). [7] Moreover *M.globosa* is the main agent causing pityriasis versicolor in tropical regions and Assam do share the same climatic condition.

This study has given as the clear insight into the mycological aspects of pityriasis versicolor and also throws a light on the predominant association of *Malassezia globosa* and *Malassezia furfur* with the disease. Eventually the confirmation of the pathogenic role of this species in pityriasis versicolor could help in understanding these conditions, which are still unclear, which promote its transformation from the saprophytic stage present in healthy skin to the parasitic one, and could also help in selecting the best therapeutic measures.

## REFERENCES

1. Crespo MJ, Abarca ML and Caban'es FJ. Atypical Lipid-Dependent *Malassezia* Species Isolated from Dogs with Otitis Externa. *J Clin Microbiol*, 2000; vol.38: 2383-2385.
2. Chaudhary R, Singh S, Banerjee T, Tilak R. Prevalence of different *Malassezia* species in pityriasis versicolor in central India. *Indian J Med Microbiol*, 2010; vol.76:159-164.
3. Byung HO, Yang WL, Yong BC & Kyu JA. Epidemiologic Study of *Malassezia*

- Yeasts in Seborrheic Dermatitis Patients by the Analysis of 26S rDNA PCR-RFLP. *Ann Dermatol*, 2010;22(2):149-155.
4. Cafarchia C, Gasser RB, Figueredo LA, Latrofa MS, Otranto D Advances in the identification of *Malassezia*, *Mol Cell Probes*, 2011 Feb;25(1):1-7
5. Crespo-Erchiga V, Ojeda A, Vera A, Crespo EA, Sanchez FF. Aislamiento e identificacion de *Malassezia* spp en pitiriasis versicolor, dermatitis seborreica y pielsana. *Rev Iberoam Micol*, 1999; 16: 516-21.
6. Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from Patients with seborrheic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol*, 2000; 38: 337-341.
7. Aspiroz C, Ara M, Varea M, Rezusta A, Rubio C. Isolation of *Malassezia globosa* and *M. Symptodialis* from patients with pityriasis versicolor in Spain. *Mycopathologia*, 2002; 154: 111-117.
8. Dutta S, Bajaj AK, Basu S, Dikshit A. Pityriasis versicolor: Socioeconomic and clinicomycological study in India. *Int J Dermatol*, 2002; 41:823-4.
9. Tarazooie B, Kordbacheh P, Zaini F, Zomorodian K, Saadat F, Zeraati H, Hallaji Z and Rezaie S. Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran. *BMC Dermatol*, 2004; 4:5-8
10. Kindo AJ, Sophia SK, Kalyani J, Anandan S. Identification of *Malassezia* species. *Indian J Med Microbiol*, 2004; 22:179-81.

How to cite this article: Agarwal SC, Sharma A, Hazarika NK et. al. Isolation And Characterization Of Different *Malassezia* Species From Pityriasis Versicolor Patients In Tertiary Care Hospital In Assam. *Int J Health Sci Res*. 2015; 5(8):213-216.

\*\*\*\*\*