

Original Research Article

A Study of Prevalence of Different Species of *Malassezia* Causing Pityriasis Versicolor and Sites of Distribution of Lesion in a Tertiary Care Hospital in Kolkata, India

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ABSTRACT

Keywords

Sites of distribution, Upper trunk, *Malassezia* sp, Pityriasis versicolor

Pityriasis versicolor is a mild, chronic superficial infection of skin, involving the stratum corneum layer and the sebaceous glands. Mostly the upper trunk is affected. The main complain of the patients being cosmetic and itchiness on profuse sweating. Most common causative agent is *Malassezia* species. Skin scrapings were collected from 108 consecutive patients from Dermatology OPD, RGKMCH. Most samples were available during summer and rainy season. Speciation done in Microbiology laboratory, RGKMCH. Direct smear preparation with 10% KOH and culture inoculation primarily in SDCA and MDA was done, followed by LCB mount and biochemical tests for speciation. In the present study, higher incidence was found in the upper trunk and the most common species being *Malassezia furfur*. Pityriasis versicolor is mostly distributed in the upper trunk. *M. furfur* to be the most prevalent species, followed by *M. globosa*, *M. restricta* and *M. sympodialis*.

Introduction

Pityriasis versicolor is a superficial skin infection caused by *Malassezia* yeasts and characterized by discrete or confluent, scaly, discoloured or depigmented areas, mainly on the upper trunk (Bigby and Casulo, 2008). The characteristic changes include hyperkeratosis, parakeratosis, acanthosis and a mild inflammatory infiltrate. They are discrete or conrescent and appear as discoloured or depigmented areas of the

skin. Lesions tend to be discrete in the beginning, but may fuse to give a serpiginous border as they enlarge.

The lesions start as tiny, multiple, macular spots that soon scale and enlarge. The lesions have often been present for more than a year when the patient is seen by a physician. Patients who seek medical attention do so for cosmetic reasons, but up to a third will have noted slight itching or

burning in the area of the lesions, particularly when sweating. The expression of infection is promoted by heat and humidity (Harrison's principle, 18th edn, p. 402). Left untreated, the infection waxes and wanes for months and years.

A common observation in reviewing a series of cases of pityriasis versicolor is that both hypo and hyperpigmented lesions occur, sometimes on the same patient.

Members of the genus *Malassezia* are opportunistic yeasts of increasing importance, due in large part to advances in detection and culture methodology which have been allowed their investigation and revealed their importance in human and animal diseases (Midgley *et al.*, 1998; Crespo-Erchiga and Gue'ho, 2005). The lipid-dependent genus *Malassezia* is considered as the causative organism of pityriasis versicolor. Of which *M. furfur*, *M. globosa*, *M. sympodialis*, *M. slooffiae*, and *M. obtusa* are considered to be the most common aetiologic agents. *M. furfur* is responsible for pityriasis versicolor mainly under tropical climate (Midgley, 2000). The differences in frequencies of *Malassezia* species among different studies may be attributed to different culture media (MDA/LNA) and perhaps to ethnic and geographic factors (Crespo-Erchiga *et al.*, 2000).

In temperate climates, the onset of disease seems to be more common from May to September (Roberts, 1969a,b). The affected areas are principally on the chest, abdomen, upper limbs, and backs (Ripon, 3rd edn, Pp. 140–145). Extensions to the thigh, neck and forearms may occur but lesions of the scalp, palms, and feet are rare. Some patients have prominent involvement of flexural surfaces, including the inguinal areas. Margins of the macules are sharply delineated.

Although the morphologic characteristics (colony & microscopic examination) for *Malassezia* is used for primary identification, but they don't provide sufficient information for specific identification of isolates. So, to avoid confusion, physiological tests should be carried out. Two simple preliminary tests for this purpose are, the subculture on SDA (at 37°C) and the catalase reaction. All lipid-dependent species except *M. restricta* exhibit a catalase positive reaction.

Materials and Methods

The study was conducted at the department of Microbiology in collaboration with the Department of Dermatology, R.G.Kar Medical College & Hospital, Kolkata-700004, during the period of June 2011 to July 2012. Patients from Kolkata and adjoining districts attending Dermatology OPD with hypo- or hyper-pigmented macular lesions were taken into consideration. Skin scrapings were collected from 108 consecutive patients from Dermatology OPD, RGKMCH. The study was prospective, cross-sectional, tertiary care hospital based. For each patient following protocol was followed:

Clinical assessment

Patients attending Dermatology OPD, RGKMCH with hypo/hyperpigmented macular lesions during June 2011 to July 2012 were examined for pityriasis versicolor. Detailed history regarding socioeconomic condition, any precipitating and aggravating factors, duration of the illness, associated itchiness were taken. Any drug intake history was also enquired about.

Specimen collection

Skin scrapings collected from the junction of active lesion and healthy and normal skin

with the help of cellotape and sterile scalpel blade.

Sample processing

All the samples were examined by 10% KOH under direct microscopy. Three skin samples were inoculated in SDCA with olive oil overlay, MDA and BA. Cultures were incubated at 37°C and 25°C on D3/D7 for any fungal growth.

Fungal growth further processed by gram staining, LCB mount (Fig. 2) and biochemical tests for species identification such as catalase and urease test, glycine assimilation and tween (20,40,60,80) utilization.

Results and Discussion

The study was carried out in the Department of Microbiology in collaboration with the Department of Dermatology, R.G.Kar Medical College and Hospital.

Cases included both hypo- and hyper-pigmented macular rashes, clinically suspected as pityriasis versicolor. Samples were collected from 108 individuals attending Dermatology OPD.

One hundred and eight samples suspected of pityriasis versicolor also showed growth of *Malassezia* on cultivation. Four *Malassezia* species were isolated among 108 culture positive isolates. *M. furfur* are highest (56.48%) in number followed by *M. globosa* (27.78%), *M. restricta* (9.26%) and *M. sympodialis* (6.48%) (Table 1). This configuration is also depicted through a pie diagram. Distribution of affected sites shows, upper trunk to be mostly affected (43.52%) and very close to it is combined type of lesion (32.4%). Only face and lower trunk is affected in 18.52% and 5.55% cases, respectively (Table 2 & Fig. 3).

Pityriasis versicolor is a mild, chronic infection of skin caused by *Malassezia* yeasts and characterized by discrete confluent, scaly, discoloured or depigmented areas (Fig. 1). The characteristic changes include hyperkeratosis, parakeratosis, acanthosis and a mild inflammatory infiltrate. The organism can be seen in the upper layers of the stratum corneum and electron microscopy shows the presence of organisms intercellularly. Increased cell turnover is found in affected cells.

The study was conducted on patients attending Dermatology OPD. The study was carried out in the department of Microbiology in collaboration with the Dermatology department, R.G.Kar Medical College and Hospital, during the midway of June 2011 to July 2012.

Both hypo- and hyper-pigmented macular rashes, clinically suspected as pityriasis versicolor were included in the study population. Samples were collected from 108 individuals attending Dermatology OPD. Out of 108 clinically suspected cases, all of which also showed growth of *Malassezia* on cultivation.

M. furfur is a member of the normal flora of skin and produces clinical disease under certain conditions that permit massive growth of the fungus. Roberts found that 97% of clinically normal people carry the fungus on the scalp and 92% carry it on their trunk (Roberts, 1969a,b). *Malassezia* infections occur in the cornified layers of the epidermis. Moderate hyperkeratosis may be seen. Plugging of the follicle followed by an overgrowth of yeast that thrives in the sebaceous environment is believed to be the aetiology. *Malassezia* yeasts require free fatty acids for survival. Usually, they are found in the stratum corneum and in the

pilar folliculi in areas with increased sebaceous gland activity such as the chest and back. The yeasts hydrolyze triglycerides into FFA and create long chain and medium chain fatty acids from free fatty acids. Healthy children less than 1yr age usually do not carry *M. furfur* on the skin (Faergemann and Fredriksson, 1980).

Pityriasis versicolor is known to affect mainly young adults (Choudhary *et al.*, 2010; Shams *et al.*, 2001, Sugita *et al.*, 2003). The commonest age group affected is 25-30 years in males and 20-25years in females but it may occur in children (El-Hefani *et al.*, 1971; Michalowski *et al.*, 1963; Miskeen *et al.*, 1984) and in the aged. This may be due to varying geographical and environmental conditions. In temperate climates, the onset of disease seems to be more common from May to September. Among the 50 Korean patients studied by Kim & Suh, 66% reported an onset between June and August. All systemic infections due to *M. furfur* or *M. pachydermatis* have been reported in patients (sply, in neonates) who were receiving IV lipid hyperalimentation (Larocco *et al.*, 1988; Long and Keyserling, 1985; Redline *et al.*, 1985). *M. furfur* has not been isolated as a saprophyte from nature or from domestic or wild animals, whereas *M. pachydermatis* has been found more commonly in animals than in humans (Salkin *et al.*, 1978; Yarrow and Ahearn, 1984).

The association of PV with malnutrition, Cushing's disease, diabetes, oral contraceptions, immunosuppression and pregnancy was frequently mentioned (Burke *et al.*, 1985; Faergemann, 1995) but in the present study we didn't find any such association.

There is also some evidence of genetically determined susceptibility. In a study it was

observed that more than one sibling in a family could get the infection. A positive family history of approximately 17% was noted in more than one study (Faergemann and Fredriksson, 1979; Krisanty *et al.*, 2009). While in spite of long intimate contact between the parents, infection found to be restricted to one of them. Workers found no higher frequency of the disease in married couples than in the general population. The present study also didn't find any familial association in the study population.

The present study conducted among 108 clinically suspected pityriasis versicolor cases revealed prevalence of four *Malassezia* species whereas in various studies most commonly isolated species are seven in number. Choudhary *et al.*, (2010) and Shams *et al.* (2001) isolated 12 *Malassezia* species of which *M. globosa*, *M. sympodialis* and *M. furfur* were of clinically significant. Present study also documented this fact. It revealed *M. furfur* to be the most prevalent species among the 108 culture positive cases. It is supported by a few studies conducted under the tropical climate (Miranda *et al.*, 2006; Aspiroz *et al.*, 2002). Next in frequency was *M. globosa* followed by *M. restricta* and *M. sympodialis*. These findings are contradictory to the studies by Aspiroz *et al.* (2002), Crespo-Erchiga *et al.* (2000), Roberts (1969a,b) and Crespo Erchiga *et al.* (2000). Dutta *et al.* (2002) who isolated *M. globosa* as the most common species at the frequencies of 58.2%, 55% and 55% respectively.

In two similar studies have been done in South India by Kindo *et al.*, (2004) revealed that *M. sympodialis* is the commonest agent (58.3%) followed by *M. globosa* (39.6%). Another study from North-Central India, conducted by Dutta *et al.* (2002) revealed that 54% of isolates belonged to *M. globosa*

and the next common species being *M. furfur* (30%).

Of the previous 17 studies, 11 showed *M. globosa* to be the most common species isolated. Its pathogenicity might be explained by high lipolytic activity (Dutta *et al.*, 2002). A few studies have found *M. sympodialis* to be the most common species.

Thus *Malassezia* species were found to be the causative organism of pityriasis versicolor. Both hypo- and hyper-pigmented

macular rashes were seen to be clinically significant.

The lesion is most prevalent in upper trunk. The present study also suggests *M. furfur* to be the most prevalent species, followed by *M. globosa*, *M. restricta* and *M. sympodialis*. Pityriasis versicolor is a superficial skin disease involving up to the stratum corneum layer of the skin and the sebaceous glands. The main complaint of the patients being cosmetic and itchiness on profuse sweating

Table.1 Prevalence of different *Malassezia* species among the total culture positive cases

TOTAL NO. OF CULTURE POSITIVE CASES	108
<i>M. furfur</i>	61(56.48%)
<i>M. globosa</i>	30(27.78%)
<i>M. restricta</i>	10(9.26%)
<i>M. sympodialis</i>	7(6.48%)

Table 1 demonstrating the prevalence of four *Malassezia* species among 108 culture positive isolates. *M. furfur* isolates are highest (56.48%) in number followed by *M. globosa* (27.78%), *M. restricta* (9.26%) and *M. sympodialis* (6.48%). This configuration is also depicted through a pie diagram.

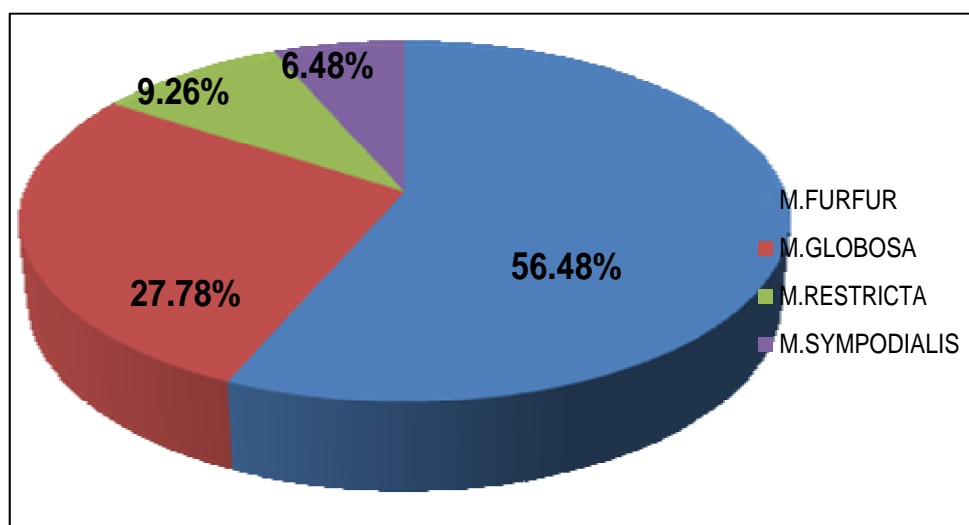


Table.2 Distribution of cases according to sites of lesion.

TOTAL NO OF CASES	ONLY FACE	UPPER TRUNK	LOWER TRUNK	COMBINED
108	20(18.52%)	47(43.52%)	6(5.55%)	35 (32.4%)

Table 2 demonstrating the distribution of affected sites; Upper trunk is mostly affected (43.52%) and very close to it is combined type of lesion (32.4%). Only face and lower trunk is affected in 18.52% and 5.55% cases, respectively.

Fig.1 Hypopigmented lesions in Pityriasis **Fig.2** LCB mount showing yeast cells of *M. furfur*

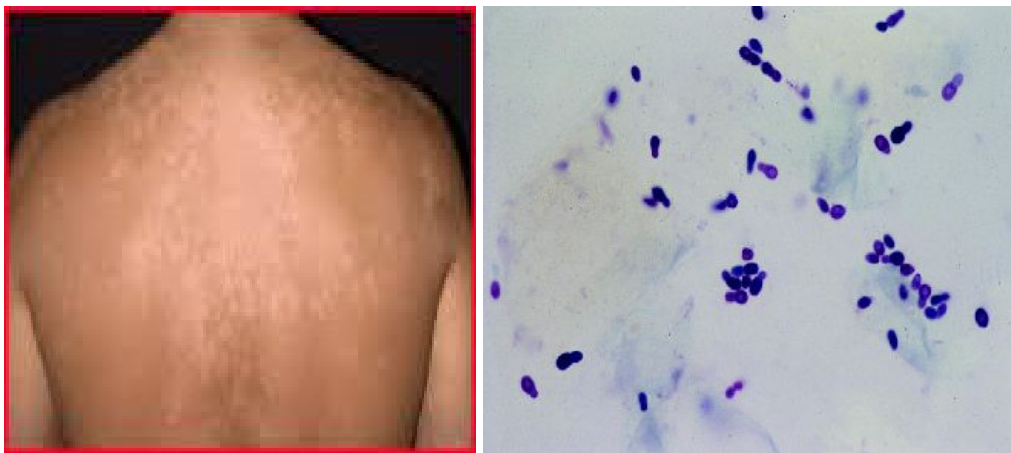


Fig.3 Distribution of pityriasis versicolor according to sites of lesion

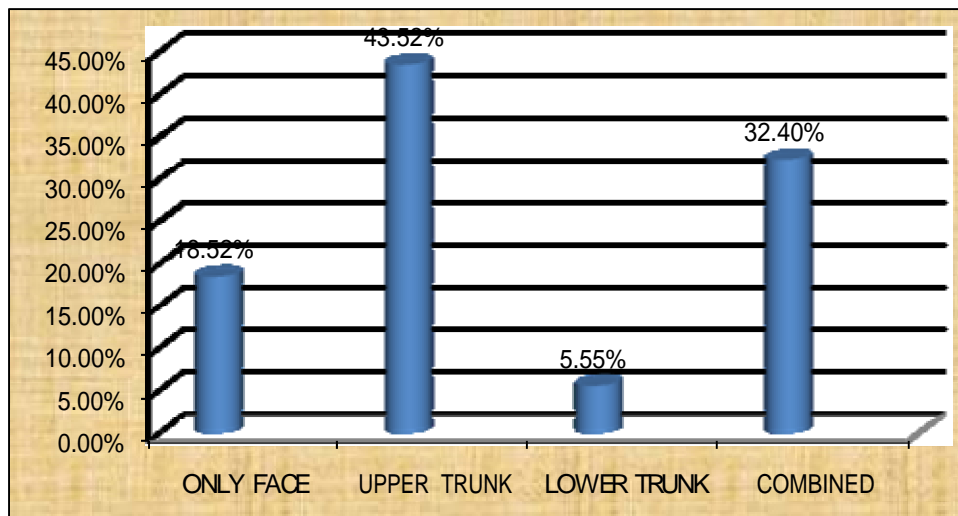


Figure 3 demonstrating the distribution of lesion according to its sites of involvement. Upper trunk is mostly affected (43.52%) followed by combined type of distribution (32.4%).

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