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Characterization and *In-vitro* Susceptibility of *Malassezia* Species In Pityriasis Versicolor Cases from a Tertiary Care Centre.

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ABSTRACT

Malassezia are common lipid dependent fungi that grow on the sebaceous areas of human skin. Although *Malassezia* are a part of the normal human skin flora, they may also cause or exacerbate several skin diseases. The aim of this study is to characterize *Malassezia* species in a tertiary care hospital in order to check the prevalence and to analyze the susceptibility pattern. Total number of samples collected was 100. KOH mount of skin scrapping was performed and samples were cultured in mDixon's agar. Speciation was done based on tween assimilation, catalase, esculin test and growth in Sabouraud's Dextrose agar. Susceptibility of *Malassezia* species to ketoconazole, fluconazole and Clotrimazole was done by disc diffusion method. Out of 100 clinically suspected cases of pityriasis versicolor, 32 yielded growth for *Malassezia* species. Among which 12 were *M. furfur*, 12 *M. globosa* and 8 *M. obtusa*. 25% of *M. furfur* & 33.3% of *M. globosa* showed resistance to ketoconazole. Though *Malassezia* species are part of normal skin flora, relapse tends to occur sooner or later in predisposed individuals and people seek treatment for cosmetic blemishes. Hence culture and antifungal susceptibility testing will help for effective treatment and prevention of relapse.

Keywords: lipophilic yeasts, *Malassezia* spp., pityriasis versicolor, tween assimilation

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INTRODUCTION

Pityriasis versicolor is a common skin complaint in which flaky discoloured patches appear mainly on the chest and back. The term 'pityriasis' is used to describe skin conditions in which the scale appears similar to bran. The multiple colours arising in the disorder give rise to the second part of the name, 'versicolor' and otherwise called 'tinea versicolor' and the causative agent is *Malassezia* (formerly known as *Pityrosporum*). It is a lipophilic fungus naturally found on the skin surfaces of many animals, including humans. [1] *Malassezia* species may also be etiological agents of skin disorders and rarely may cause systemic infections. The genus has been recently expanded to include thirteen species [1], namely *M. globosa*, *M. restricta*, *M. sympodialis*, *M. furfur*, *M. obtusa*, *M. slooffiae*, *M. pachydermatis*, *M. dermatis*, *M. japonica*, *M. nana*, *M. equina*, *M. caprae* and *M. yamatoensis* but their contribution to skin diseases is under investigation. They require an exogenous source of specific lipids to grow in culture. They have been reported as agents of more invasive human diseases including deep-line catheter-associated sepsis. The latter infection occurs in patients, primarily infants, receiving parenteral nutrition (including lipid emulsions) through the catheter. The lipids presumably provide growth factors required for replication of the organisms[12].

Evidence that these yeasts require lipids comes from their ability to produce lipases. These lipases are involved in the release of arachidonic acid, which may be involved in cutaneous inflammation. Pityriasis versicolor is diagnosed by its clinical appearance and the observation of many yeast cells and hyphae in scales stained with methylene blue or Potassium Hydroxide mount (KOH) in microscopic examination. Due to the lipid dependence of the organism, sugar fermentation tests are not appropriate for species differentiation. At present, all species are identified according to colony morphology, microscopic characteristics and complicated lipid assimilation tests.[2] Despite these major taxonomic revisions, little work has subsequently been published about the in vitro susceptibilities of these species to various antifungal agents.[4] *M. furfur* is readily killed by almost all topical and systemic antifungals [6] but since the organism is part of the normal flora on human skin, it is impossible to eradicate it permanently. As a consequence, relapse tends to occur sooner or later in predisposed individuals.[6] Hence, the present study is to characterize *Malassezia* species in a tertiary care hospital in order to check the prevalence and to analyze the susceptibility pattern.

MATERIALS AND METHODS

During the period of six months from August 2011 to January 2012, 100 samples were collected from the clinically suspected patients with pityriasis vesicular attending Dermatology out-patient department and the samples were transported to Mycology section of Microbiology department. After entering the details in the proforma, samples were processed as follows.

Direct Microscopy

All the skin scrapings were subjected for Microscopic examination by KOH mount to detect the presence of hyphae and spores, which generally exhibit the characteristic appearance of “Spaghetti and Meatballs.”

Culture

The scales were inoculated into modified Dixon’s agar (mDixon’s agar) as described by Guillot *et al.*[7] and into Sabouraud dextrose agar containing 0.05% chloramphenicol and 0.05% cycloheximide (SDA). The tubes were incubated at 32°C and checked every day for the suspected growth of *Malassezia* upto seven days.

Microscopy of the culture: Once there was growth in the medium, Gram staining was done which showed “Pop bottle or bottle neck appearance” of yeast form of *Malassezia*.

Speciation

Once the colonies had grown, it was speciated by performing lactophenol cotton blue mount, urease test, catalase test, esculin hydrolysis and temperature tolerance test. Utilization of Tween 20, 40, 60 and 80 was detected by a standard procedure and based on the results, speciation was done for *Malassezia* (Table I) [16]

Antifungal susceptibility testing

For Ketoconazole(10µg), Clotrimazole(10µg) and Fluconazole(25µg) was analysed by disk diffusion method following the methodology of Naeini et al[10].

RESULTS

Out of 100 samples received, 56 were obtained from male and 44 from female. Among these 56 males, 19 yielded culture positive and from 44 females 13 yielded culture positive.

Table 2 shows that out of 100 samples, 39 (39%) were between the age group 21-30 years.

Among the 100 Pityriasis versicolor patients, 83 showed hypopigmentation and 17 showed Hyperpigmentation.

Table 3 shows the distribution of site of infection where 66% patients had lesions predominantly on trunk.

Out of 100 samples, 41 were KOH positive and 32 were culture positive. Fig 1 shows bottle neck appearance of *Malassezia* in Gram staining. Fig 2 shows the colony morphology of *Malassezia* species on mDixon’s agar

<i>Malassezia</i> species	Lipid dependence	growth rate >37°C	Catalase	Esculin	Cremophor	Tween		
						20	40	80
<i>M. furfur</i>	+	+	+	-	V	+	+	+
<i>M. pachydermatis</i>	-	+	V	V	V	+	+	+
<i>M. sympodialis</i>	+	+	+	+	-	-	+	+
<i>M. globosa</i>	+	-	+	-	-	-	-	-
<i>M. obtuse</i>	+	-	+	+	-	-	-	-
<i>M. restricta</i>	+	-	-	-	-	-	-	-
<i>M. slooffiae</i>	+	+	+	-	-	+	+	-

Table 1- Scheme for Identification of *Malassezia* species

Table 2-Age and sex wise distribution

	0-10	11-20	21-30	31-40	41-50	>51	Total
Male	4	12	22	12	3	3	56
Female	4	4	16	9	10	1	44
Total	8	16	38	21	13	4	100

Table 3- Site of distribution

SITE OF LESION	PERCENTAGE
TRUNK	66%
FACE	17%
NECK	15%
SCALP	2%

Table 4 – Distribution of *Malassezia* species

MALASSEZIA SPECIES	DISTRIBUTION
<i>M. furfur</i>	37.5%
<i>M. globosa</i>	25%
<i>M. obtuse</i>	37.5%

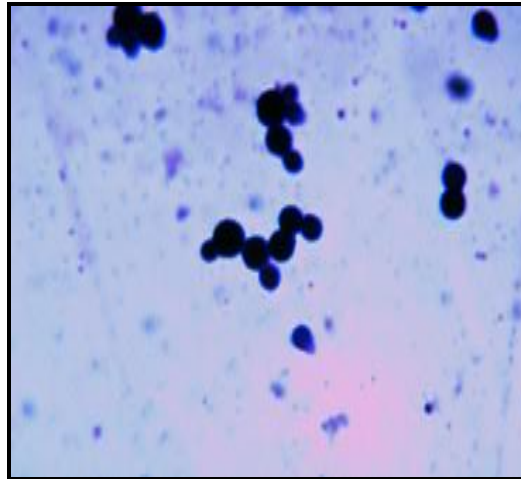


Fig: 1 *M. globosa* - Gram staining



Fig:2 Colony morphology of *Malassezia* species

Among the 32 culture positive patients, 23 (69%) were found to have recurrent infection.

Tween assimilation, one of the procedures for speciation of *Malassezia* species is shown in fig 3 and Fig 4.

Table 4 shows the distribution of *Malassezia* species in which the predominant isolates were *M. furfur*, and *M. globosa* each 12 (37.5%) followed by *M. obtusa* 8 (25%).

Among the 12 isolates of *M. Furfur*, 75% were sensitive to ketoconazole, 100% of the isolates were susceptible to fluconazole and clotrimazole which is shown in Fig 5. Out of 6 strains of *M. globosa*, 100% were susceptible to fluconazole & clotrimazole and 66.7% were sensitive to ketoconazole. All the isolates of *M. obtusa* were sensitive to clotrimazole, ketoconazole and fluconazole.

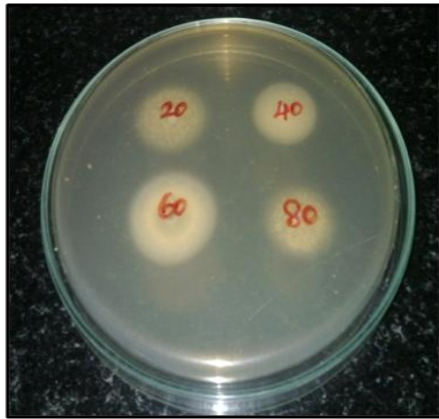


Fig 3 Tween assimilation – *M.furfur*



Fig 4 Tween assimilation - *M.obtusa*

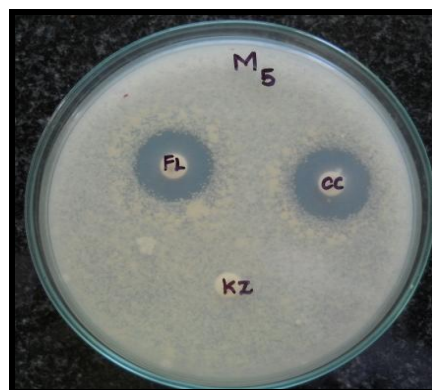


Fig 5 Antifungal susceptibility pattern of *M.furfur*: ketoconazole

DISCUSSION

In this study, most of the patients were between the age group 21-30 (39%) followed by 31-40 (21%). A similar study by *Shokohi et al*, [13] showed most of the patients belonged to the age group of 21-30 years (36%). This could be explained by the fact that sebum production is at its peak in this age group. There were 56 males and 44 females in this study. Male preponderance seen in the present study may be due to the fact that they are more involved with outdoor activities, which place them at high risk of exposure to factors like high temperatures and humidity.

In our study, out of the 100 specimens that were inoculated on mDA, 32% yielded growth of *Malassezia*. Out of this, the most predominant isolates were *Malassezia furfur*, 12 (37.5%), followed by *M. globosa*, 12 (37.5%) followed by 8 isolates of *M. obtusa* (25%). However, in a study by *Rezvan Moniri et al* [8], *M. Globosa* (43.8%) was the predominant

isolate followed by *Malassezia Furfur* (38.4%), *M. Obtusa* (9.8%), *M. Sympodialis* (6.3%), and *M. Slooffiae* (1.7%).

In our study, out of 32 culture positive patients 23 (69%) were found to have history of recurrence of infection. 48% recurrence was documented by *Ghosh et al* [14] and *Krishnan et in al.* [15] in their study

Among the 12 isolates of *M. Furfur*, 75% were sensitive to ketoconazole and 100% of the isolates were susceptible to fluconazole & clotrimazole. Out of 6 *M. globosa*, 100% were susceptible to fluconazole & clotrimazole and 66.7% were sensitive to ketoconazole. Among 3 isolates of *M. obtusa* 100% were sensitive to clotrimazole, ketoconazole and fluconazole whereas *Margarita Garau* [4] reported that all the strains were resistant to 5FC(Fluconazole).

In a related study by *A.K.Gupta et al* [11] all strains of *Malassezia* species isolated in their study were susceptible to ketoconazole, voriconazole and itraconazole at low concentrations.

To conclude, the procedure of culture and antifungal testing is required to be performed as different species of *Malassezia* are involved in Pityriasis versicolor and susceptibility is different among different species. Thus, it would help to prevent recurrences and any systemic complications

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