

Original Article

Identification of *Malassezia* species from suspected Pityriasis (versicolor) patients

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Abstract

Pityriasis versicolor is a chronic, superficial fungal infection affecting the superficial layer of a stratum corneum. *Malassezia furfur* is the major species involved in pityriasis versicolor. Currently many researchers reported increase in the incidence of other species as a causative agent of pityriasis versicolor. Isolation and identification of *Malassezia* species from suspected Pityriasis versicolor patients was conducted in the Department of Microbiology and immunology Bangabandhu Sheikh Mujib Medical University (BSMMU) from September 2013 to August 2014. Ninety two clinically diagnosed patients of Pityriasis versicolor were studied and samples from skin lesion were processed for direct microscopy and culture. Species of *Malassezia* were identified by cultural characteristics in Dixon's agar media by macro and microscopic observation of the colonies and by catalase test, urease test, esculin test and tween assimilation test. A total of 92 cases 70(70.08%) were positive by direct microscopy and 50(54.34%) were positive by culture. *Malassezia globosa* was found in 38(76%) cases as the commonest etiological agent and *Malassezia furfur* was found in 10(20%) cases and *Malassezia obtusa* in 2 (4%) cases respectively.

Key words: Dixon's agar media, *Malassezia* spp, Pityriasis versicolor.

Introduction

Pityriasis versicolor is a chronic, benign skin disease that is generally asymptomatic. It occurs worldwide and is very common in tropical and temperate regions.^{1,2} It predominately affects young adults of both genders. In several studies on the basis of morphology, ultra structure, physiology and molecular biology the genus *Malassezia* includes the following species: *Malassezia globosa*, *Malassezia furfur*, *Malassezia restricta*, *Malassezia obtusa*, *Malassezia slooffiae*, *Malassezia sympodialis*, *M. yamatoensis*, *M. dermatis*, *M. nana* and *M. japonica*.^{7, 8, 9, 10, 11}

A study conducted by Kashan et al¹² in Iran reported out of 118 clinically suspected specimens of pityriasis versicolor found 114(96.61%) cases positive for microscopy and out of these 114 microscopy positive cases 105 (92.10%) were positive for culture. Similar study conducted by Aggarwal et al¹³ in India in 2013, with 65 patients found 58(89.23%) cases microscopy positive and out of 58 microscopic positive case

54(93.10%) were positive for culture. Shah et al¹⁴ in India in 2013, reported *M. globosa* in 48.57% cases and *M. furfur* in 34.28% cases. Guisino et al¹⁷ in Argentina 2009 also reported *Malassezia sympodialis* in 37.7% and *Malassezia globosa* in 37.2%, *M. furfur* in 21.3%, *M. slooffiae* in 1.7%, *M. restricta* in 1.3% and *M. dermatis* in 0.4% cases. Petry et al¹⁸ in Brazil reported *M. sympodialis* in 30% cases followed by *M. furfur* in 25.75%, *M. globosa* in 22.7%, *M. restricta* in 12.1%, *M. obtusa* in 7.6% and *M. slooffiae* in 1.5% respectively. *Malassezia globosa* has been reported as the main causative agents among Pityriasis versicolor patients studies in India, Iran and Sri Lanka respectively.^{14, 15, 16} In a study by Krisanty et al²² in Indonesia found *M. furfur* in 42.9%, *M. sympodialis* in 27.5%, *M. globosa* in 13.3%, *M. obtusa* in 7.7% and *M. restricta* in 2.2% cases.

Malassezia furfur was considered the main etiological agent of Pityriasis versicolor for a long time. However, this changed during the last decade as research to further investigate the distribution of this fungus in affected humans revealed a predominance of other species such as *Malassezia globosa*.²³ *Malassezia globosa* was found to be a species with high level of esterase and lipase enzyme activity probably. These enzymes play an important role in pathogenicity.²⁴

Data regarding antifungal sensitivity patterns vary from species to species and data are very few. In vitro susceptibility

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studied by researchers have shown variations in susceptibility of different Malassezia species to various antifungal agents. Strains of *M.furfur*, *M. globosa* and *M. obtusa* have been found to be more tolerant to Terbinafine then *M. restricta*, *M. sympodialis* species²⁵. Species involved in Malassezia infection and their antifungal susceptibility test are necessary to determine which species are implicated in Pityriasis versicolor. Variation in susceptibility of Malassezia species to various antifungal agents is also necessary to determine for the proper treatment of the patient.

So this study was aimed to isolation and identification of Malassezia species from patients with Pityriasis versicolor.

Materials and Methods

This was carried out in the Department of Microbiology and Immunology,BSMMU, Shahbagh, Dhaka, Bangladesh from September 2013to August 2014. Informed written consent was taken from the patients. Scraping from skin lesion of 92 clinically suspected Pityriasis versicolor patients attending outpatient, department of Dermatology and Venereology, BSMMU. All the laboratory tests were done in the department of Microbiology and Immunology,BSMMU. A portion of the sample was used for direct microscopic examination with 20% KOH and the remaining portion was used for culture in Dixon's agar and SDA with olive oil media and then incubated at 32°C for 14 days.¹⁶

Culture plates were examined on days3 and 7 then weekly intervals up to two weeks.¹⁵ Then confirm diagnosis of Malassezia species were done by their gross colony morphology, microscopic characteristics by Gram stain and catalase test, urease test, esculin test and Tween assimilation test.²⁰ Then all data were analyzed by using computer based SPSS (Statistical Package of social science) software version 20. P -value measured by Chi-Square test.

Results

Ninety two clinically suspected Pityriasis versicolor patients were enrolled in this study. Majority of the patient were in 21-40 years age group (Table-I). out of 92 study population 70(76.08%) were positive for Malassezia by microscopy and 50(54.34%) by culture and 50(54.34%) were positive by both microscopy and culture.Out of 70 microscopy positive case 20(28.57%) were negative by culture.All culture positive cases were positive by microscopy but of the 42 culture negative cases 20 case were positive by microscopy. The difference between microscopy and culture were statistically significant (Table-2).Among 50 isolated *Malassezia* from culture, *M. globosa* were found in 38(76%) cases followed by *M. furfur* in 10 (20%) and *M. obtusa* in 2(4%) cases. *Malassezia globosa* is the predominant species (Table-3).

Table 1: Age and sex distribution of the study population (n=92)

Age group	Male N. (%)	Female N. (%)	Total N. (%)
10-20	5 (5.43)	3 (3.26)	8 (8.69)
21-30	45 (48.91)	4 (4.34)	49 (53.26)
31-40	30 (32.60)	2 (2.17)	32 (34.78)
41-50	2 (2.17)	1 (1.08)	3 (3.26)
Total	82.(89.11)	10 (10.85)	92. (100)

Table 2: Microscopic findings and culture results of the study population (n=92)

Examination method	Positive for <i>Malassezia</i>	Negative for <i>Malassezia</i>
Microscopy	70 (76.08%)	22(23.91%)
Culture	50 (54.34%)	42(45.65%)
Microscopy and Culture positive	50 (54.34%)	42(45.65%)
Microscopy positive and Culture negative	20 (21.73%)	72(78.26%)

Table 3: Species distribution of isolated Malassezia in the study population (n= 50)

Species of <i>Malassezia</i>	Number	Percentage (%)
<i>M. globosa</i>	38	76
<i>M. furfur</i>	10	20
<i>M. obtuse</i>	2	4
Total	50	100

Discussion

Malassezia yeasts are lipophilic fungi which are normal flora of the skin and are recovered in 75-98% of healthy individuals.²¹This study was carried out to identify Malassezia species isolated from suspected Pityriasis versicolor patients. Most of the patients (89.11%) were male in our study. Shah et al 2013¹⁴ in central India and Moniri et al 2009¹²in Iran reported 59.71% male in and 64.4% respectively. Kalyani et al 2014¹⁹ in India reported 41% microscopy positivity and 32% culture positivity and Moniri et al 2009¹² in Iran reported 96.6% positivity by microscopy and by culture 92%. Aggarwal et al¹³ in Punjab, India found 89.23% positive by microscopy and 93.10% by culture. These findings are 28.57% microscopy positive sample were negative in culture. But in our study all culture positive cases were positive by microscopy and microscopy yielded higher positivity than culture. Shah et al¹⁴ in Channi, India reported *M. globosa* in 48.7% case. *M.furfur* in 34.28%

cases. Chaudhury *et al*¹⁵ in central India found *M. globosa* in 43.8% cases and *M. furfur* 38.4% cases. Moniri *et al*¹² in Kashan, Iran reported *M. globosa* in 43.8% cases and *M. furfur* in 38.4% cases. The differences in species between various studies could be due to ethnic and geographical factors, may be influenced by use of different sampling techniques and the use of different culture media.⁴

Direct microscopy (KOH preparation) is still the main stay of diagnosis of *Malassezia*. Culture is necessary for species identification and determination of antifungal sensitivity. Dixon's agar media could be used for identification of *Malassezia* species and also it could be used for primary culture from clinical sample.

References

- Gupta AK, Batra R and Dawson T *et al*. Skin disease associated with *Malassezia* species. *J Am Acad Dermatol* 2004;51:785-98.
- Gupta AK, Kohli Y and Faergemann *et al*. Epidemiology of the *Malassezia* yeast associated with Pityriasis Versicolor in Ontario, Canada. *Med Mycol*.2001; 39:199-06.
- Ashbee HR, Evans EG. Immunology of diseases associated with *Malassezia* species. *Clinical Microbiol Rev*.2002; 15: 21-27.
- Crespo Erchiga V, Delgado Florencio V. *Malassezia* species in skin diseases. *Curr Opin Infect Dis*.2002;15: 133-42
- Gueho E, Ashbee HR and Guillot *et al*. The role of *Malassezia* species in the ecology of human skin and as pathogen. *Med Mycol*.1998; 36:220-29.
- Gupta AK, Bluhm R and Summerbell *et al*. Pityriasis Versicolor. *J Eur Acad Dermatol Venerol*.2002;16:19-33.
- Gueho E, Midgley G and Guillot *et al*. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek*.1996; 69:337-55.
- Hirai A, Kano R and Makimura K *et al*. *Malassezia nana* species, a novel lipid dependent yeast species isolated from animals. *Inter. J Syst Evol Microbiol*.2004;54:623-27.
- Sujita T, Tajima M and Saito M *et al*. A new yeast *Malassezia yamatoensis* isolated from patient with seborrheic dermatitis and its distribution in patients and healthy subjects. *Microbiol Immunol*.2004; 48: 576-83.
- Sujita T, Takshima M and Nishikawa A *et al*. A description of a new yeast species *Malassezia japonica* and its detection in patients with atopic dermatitis and healthy subjects. *J Clin Microbiol*.2003;41:4695-99.
- Sujita T, Suto H and Unno T *et al*. New yeast species *Malassezia dermatis* isolated from patients with atopic dermatitis. *J Clin Microbiol*.2002;40:1363-67.
- Moniri R, Nazeri M and Amiri S *et al*. Isolation and identification of *Malassezia* spp. in Pityriasis versicolor in Kashan, Iran. *Pakistan J Med Science*. 2009;25 (5) 837-40.
- Aggarwal P, Bala M and Manhas A *et al*. Study of distribution of *Malassezia* species in patients with Pityriasis versicolor and healthy individuals in tertiary care hospital, Punjab. *Indian Journal of Medical Microbiology*.2013; 31(3):270-74.
- Shah A, Koticha A and Khopkar U *et al*. Identification and speciation of *Malassezia* in patients clinically suspected of having Pityriasis versicolor. *Indian J Dermatol*.2013; 58(3): 239.
- Chaudhary R, Singh S and Tilak R *et al*. Prevalence of different *Malassezia* species in Pityriasis versicolor in central India. *Indian J Dermatol Venerol, Leprol*.2010; 76 :159-6.
- Sandra R, Adriana C and Crespo de G *et al*. *Malassezia* yeast species isolated from patients with dermatologic lesions. *Biomedica*.2005; 25:189-95
- Giusiano G, Rojas F and Mangiaterra M *et al*. Prevalence of *Malassezia* species in Pityriasis versicolor lesions in northeast Argentina. *Micol*.2010;27(2): 71-74.
- Petry V, Weiss L and Milan T *et al*. Identification of *Malassezia* yeast species isolated from patients with Pityriasis versicolor in Brazil. *An Bras Dermatol*,2011; 86(4):803-6.
- Kalyani M, Shameem AS and Jayakumar *et al*. Characterization and In vitro susceptibility of *Malassezia* species in Pityriasis versicolor cases from a tertiary care centre, Tamilnadu, India. *Research Journal of Pharmaceutical, Biological and Chemical science*.2014; 5(1):585.
- Evelin Gueho-Killermann and Teun Boekhout. Biodiversity, Phylogeny and Ultrastructure of *Malassezia* yeast. Springer Verlag Berlin Heidelberg.2010;17-63.
- Afshar P, Ghasemi M and Kalhori S *et al*. Identification of *Malassezia* species isolated from patients with Pityriasis versicolor. *Jundishapur Journal of Microbiology*.2012;6(6):8581.
- Krisanty RI, Bramono K and Wisnu I *et al*. Identification of *Malassezia* species from Pityriasis versicolor in Indonesia and its relationship with clinical characteristics. *Mycoses*. 2009;52(3):257-62.
- Santana JO, Pedro Costa and Filho *et al*. Clinical epidemiological characterization of patients in the urban area of Buerarema BA, Brazil.2013;88(2):216-21.
- Aspiroz C, Varea M and Rubio C *et al*. Isolation of *Malassezia globosa* and *M. sympodialis* from patients with Pityriasis versicolor in Spain. *Mycopathologia*.2010; 153:111-17.
- Mandell, Douglas and Benett. Principle and practice of infectious diseases 6th ed. Elsevier Philadelphia, USA.2005 ;(3):2383-84.