

## ISOLATION AND IDENTIFICATION OF MALASSEZIA SPP. IN *PYTIRIASIS VERSICOLOR* IN KASHAN, IRAN

Rezvan Moniri<sup>1</sup>, Mehdi Nazeri<sup>2</sup>, Shokouh Amiri<sup>3</sup>, Babak Asghari<sup>4</sup>

### ABSTRACT

**Objective:** Pityriasis versicolor (PV) is a chronic superficial fungal disease caused by *Malassezia* spp. The incidence is as high as 30-40% in tropical climates. Epidemiological data suggest geographical variations in the rate of the isolated species from PV. Our aim was to identify *Malassezia* spp. from PV patients in Kashan, Iran.

**Methodology:** Isolates of *Malassezia* were collected from 118 PV patients (75 males and 43 females). A direct microscopy with KOH and methylene blue was carried out. Cultures were made in modified Dixon agar medium and the isolates were identified by macroscopic and microscopic features, physiological characteristics (catalase test) and biochemical criteria (esculin and lipid assimilation tests). Data were analyzed statistically by software SPSS (version 11) and Fischer's exact and descriptive statistical tests.

**Results:** The average age of 118 patients in this study was 28.42±8.53 years. The percentages of patients in this study were 64.4 and 35.6 for men and women respectively. Hyperhidrosis was reported as the most important finding with 58.1%. Back (42.2%) and extremities (7.4%) were the highest and the lowest involved parts respectively. The isolates found were *M. globosa* (43.8%), *Malassezia furfur* (38.4%), *M. obtusa* (9.8%), *M. sympodialis* (6.3%), and *M. slooffiae* (1.7%).

**Conclusion:** From these findings it was suggested that *M. globosa* presents the main species implicated in the pathogenicity of PV and *M. furfur* as the second agent of importance.

**KEY WORDS:** *Malassezia* spp, Pityriasis versicolor.

Pak J Med Sci October - December 2009 (Part-I) Vol. 25 No. 5 837-840

### How to cite this article:

Moniri R, Nazeri M, Amiri S, Asghari B. Isolation and identification of *Malassezia* spp. In pityriasis versicolor in Kashan, Iran. Pak J Med Sci 2009;25(5):837-840.

### INTRODUCTION

*Pityriasis versicolor* (PV) is a superficial infection of the stratum corneum caused by *Malassezia* spp.,<sup>1</sup> pathological change occurred by inocu-

lating them onto human skin, and they were detected from the pathological change.<sup>2</sup> PV varies depending on tropical or temperate climate. Patients with PV increase in summer. PV is observed in the 20 to 30 year old group, but is uncommon in children and the elderly.<sup>3,4</sup> PV is not uncommon among children in the tropics.<sup>5</sup> PV is diagnosed by its clinical appearance and the observation of many yeast cells and hyphae in scales stained with methylene blue or KOH in microscopic examination. *Malassezia* species are lipophilic yeasts and are considered part of the normal flora of the skin, being particularly common on the scalp, face and trunk. Sei et al.,<sup>6</sup> reported that the number of *Malassezia* species

---

#### Correspondence

Rezvan Moniri,  
Associate Professor of Microbiology,  
Dept. of Microbiology and Immunology,  
Kashan University of Medical Sciences,  
P.O.Box: 87155-111  
Kashan - Iran.  
E-mail: moniri@kaums.ac.ir

- \* Received for Publication: December 3, 2008
- \* Revision Received: July 18, 2009
- \* Revision Accepted: July 24, 2009

on the face increased for one month after birth, and started to decrease four months later in parallel with the amount of sebum. Until recently, *M. furfur*, *M. pachydermatis*, and *M. sympodialis* were the only members of the genus, but in 1996, Gue'ho et al. added four new species, *M. globosa*, *M. obtusa*, *M. restricta*, and *M. slooffiae*, based on their morphology, ultra-structure, physiology, and molecular biology.<sup>7</sup> Recently, four new species were isolated, namely *M. dermatis*,<sup>8</sup> *M. japonica*,<sup>9</sup> *M. yamatoensis*<sup>10</sup> and *M. nana*<sup>11</sup> in Japan. *Malassezia* species are associated with PV, *Malassezia* folliculitis, seborrheic dermatitis (SD), dandruff, atopic dermatitis (AD)<sup>12</sup> and CRP.<sup>13</sup> Research attention has focused on the relationship between *Malassezia* species and skin disease. Until recently, PV was a superficial infection believed to be caused by *M. furfur*. Recent studies<sup>14-16</sup> using morphological features and physiological tests indicate that *M. globosa* may actually be the causative agent, but the *M. globosa* rate of culture changed with reporters from 53 to 97%. On the other hand, Gupta et al.,<sup>17</sup> reported that *M. sympodialis* was detected at 60%. Nakabayashi<sup>18</sup> reported that *M. globosa* was isolated from 55% of lesional skin specimens in PV, while other species were below 10% according to the method devised by Guillot et al.<sup>19</sup> The present study was aimed to identify the *Malassezia* spp. causing pityriasis versicolor in a Kashan population in Iran.

## METHODOLOGY

This descriptive study was conducted from August 2006 to October 2007. The samples were collected from patients with pityriasis versicolor in Kashan, Iran and processed in the mycology laboratory of the department of microbiology. Mycological evaluation by microscopic examination of KOH treated skin scrapings and methylene blue staining were done and 118 were cultured. The scales were inoculated into modified Dixon's agar (mDixon's agar) as described by Guillot *et al* and into Sabouraud dextrose agar containing 0.05% chloramphenicol and 0.05% cycloheximide (SDA). The tubes were incubated at 32°C for 3-4 days. The morphology of the

yeast cells was studied by making Gram stained smears of the isolates from mDixon's agar after one week incubation at 32°C. Ethical approval for the study was obtained from Research and Ethics Committee of the Kashan University of Medical Sciences.

## RESULTS

The average age of 118 patients (76 males and 42 females) in this study was 28.42±8.53 years. Characteristics of patients with pityriasis versicolor in Kashan, Iran according to sex and age group shown in Table-I.

One hundred fourteen (94.2%) skin scrapings showed hyphae and spores in the KOH preparation. Growth was obtained on mDixon's agar 112 out of 121(92.6%) skin scrapings. The isolates found were *M. globosa* (43.8%), *Malassezia furfur* (38.4%), *M. obtusa* (9.8%), *M. sympodialis* (6.3%), and *M. slooffiae* (1.7%). There was no statistically significant association between *Malassezia* spp. and demographic characteristics and clinical characteristics of the patients. Hyperhydrosis was reported as the most important finding with 58.1%. The frequency rate of patients with pityriasis versicolor according to site of lesion are shown in Table - II.

## DISCUSSION

In our study, out of the 118 specimens that were inoculated, 92.6% yielded growth of *Malassezia* in culture. Out of this, the most frequently isolated species was *Malassezia globosa* (43.8%), followed by *M.furfur* (38.4%), *M. obtusa* (9.8%), *M. sympodialis*(6.3%) and *M.*

Table-I: Characteristics of patients with pityriasis versicolor in Kashan, Iran according to sex and age group

Age group	Female No. (%)	Male No. (%)	Total No. (%)
15 < years	2(4.8)	2(2.6)	4 (3.4)
16-30	36(85.6)	45((59.2)	81 (68.6)
31-45	2(4.8)	23(30.3)	25 (21.2)
>45	2(4.8)	6(7.9)	8 (6.8)
Total	42(100)	76(100)	118 (100)

Table-II: The frequency rate of patients with pityriasis versicolor according to site of lesion

Back	42.2%
Chest	38%
Face	12.4%
Extremities	7.4%

*slooffiae* (1.7%). 42.2% of isolates, predominating in the back skin.

In an earlier study, Crespo *et al* reported that *M.globosa* was recovered from 97% of their patients, alone in 60% of them and associated with *M.sympodialis* in 29% and *M. slooffiae* in 7%.<sup>14</sup> Crespo Erchiga *et al.* showed that in pityriasis versicolor, *Malassezia globosa* was found in 84% of cases, alone or associated with *Malassezia sympodialis*, which was by far the commonest species in normal skin (91.7% of isolates, predominating in the trunk skin).<sup>20</sup> Kindo *et al.*, described that out of 70 scrapings 48 (68.75%) showed growth on mDixon's agar. The commonest isolate was *M. sympodialis* (28, 58%) followed by *M. globosa* (19, 40%) and one isolate was (2%) of *M. restricta*.<sup>21</sup> Salah *et al.* reported that *Malassezia globosa* was the predominant species in lesional skin of PV (65%). It was isolated alone in 47% of cases and associated in 18% with *M. furfur* (13%) or *M. sympodialis* (5%). In healthy skin *M. globosa* was found alone in 7.77% and associated in 15.54%, respectively, with *M. furfur* (4.44%), *M. sympodialis* (4.44%), *M. restricta* (3.33%) and *M. slooffiae* (1.11%).<sup>22</sup> Crespo-Erchig *et al.* indicated that *Malassezia globosa* is the predominant species found in the lesions of pityriasis versicolor, at least in temperate climates.<sup>23</sup>

In a study by Krisanty *et al.* the isolates found were *Malassezia furfur* (42.9%), *M. sympodialis* (27.5%), *M. globosa* (13.3%), *M. slooffiae* (7.7%), *M. obtusa* (7.7%) and *M. restricta* (2.2%), and 7.14% specimens were unidentified.<sup>24</sup> Karaka<sup>o</sup> *et al.* reported that 45.4% of the patients showed *Malassezia* spp. in culture and *Malassezia globosa* (47.7%) was the most commonly isolated species followed by *Malassezia furfur* (36.4%) and *Malassezia slooffiae* (15.9%).<sup>25</sup> In this study, the most common isolated species in PV lesions

Table-III: The results of growth on mDixon's agar and microscopic examination from 121 specimens

Growth on mDixon's agar	Positive No. (%)	Negative No. (%)	Total No. (%)
Microscopic examination			
Positive	105(86.7)	9(7.4)	114(94.2)
Negative	7(5.7)	0(0)	7(5.8)
Total	112(92.6)	9(7.4)	121(100)

was *M. globosa*, which is in agreement with the majority of studies worldwide.<sup>20,22,23,25,26</sup> This was contrary to observation of Krisanty *et al.*, which isolated *M. furfur* and *M. sympodialis* as the predominant species in PV lesions.<sup>24</sup>

From these findings it was suggested that *M. globosa* presents the main species implicated in the pathogenicity of pityriasis versicolor and *M. furfur* as the second agent of importance, and predominating site of infection was in the back skin.

#### ACKNOWLEDGEMENTS

The financial support of the Kashan University of Medical Sciences and Health Services is gratefully acknowledged. We are also grateful to Dr. Morraveji for his valuable advice in this study. *Conflict of interest:* No competing interest declared.

#### REFERENCES

- Morishita N, Sei Y, Sugita T. Molecular analysis of *Malassezia* microflora from patients with pityriasis versicolor. *Mycopathologia* 2006;161:61-65.
- Faergemann J, Frediksson T. Experimental infection in rabbits and humans with *Pityrosporum orbiculare* and *P. ovale*. *J Invest Dermatol* 1981;77:314-318.
- Kasai T. Epidemiological investigation committee for human mycoses in the Japanese society for medical mycology. 1997 epidemiological survey of dermatophytoses in Japan. *Nippon Ishinkin Gakkai Zasshi* 2001;42:11-18.
- Tan HH. Superficial fungal infections seen at the National Skin Centre, Singapore. *Nippon Ishinkin Gakkai Zasshi* 2005;46:77-80.
- Jena DK, Sengupta S, Dwari BC, Ram MK. Pityriasis versicolor in the pediatric age group. *Ind J Dermatol Venereol Leprol* 2005;71:259-261.
- Sei Y, Nakabayashi A, Morishita N, Takiuchi I. Infantile Seborrheic Dermatitis-The etiology and role of *Malassezia furfur*. *J Pediatr Dermatol* 2000;19:101-104.

7. Gue'ho E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek* 1996;69:337-355.
8. Sugita T, Takashima M, Shinoda T. New Yeast Species, *Malassezia dermatis*, Isolated from Patients with Atopic Dermatitis. *J Clin Microbiol* 2002;40:1363-1367.
9. Sugita T, Takashima M, Kodama M. Description of a New Yeast Species, *Malassezia japonica*, and its Detection in Patients with Atopic Dermatitis and Healthy Subjects. *J Clin Microbiol* 2003;41:4695-4699.
10. Sugita T, Tajima M, Takashima M. A New Yeast, *Malassezia yamatoensis*, isolated from a Patient with Seborrheic Dermatitis, and Its Distribution in Patients and Healthy Subjects. *Microbiol Immunol* 2004;48:579-583.
11. Hirai A, Kano R, Makimura K. *Malassezia nana* sp Nov, a novel lipid-dependent yeast species isolated from animals. *Int J Syst Evol Microbiol* 2004;54:623-627.
12. Gupta AK, Batra R, Bluhm R. Skin disease associated with *Malassezia* species. *J Am Acad Dermatol* 2004;51:85-798.
13. Stein JA, Shin HT, Chang MW. Confluent and reticulated papillomatosis associated with tinea versicolor in three siblings. *Pediatr Dermatol* 2005;22:331-333.
14. Crespo EV, Ojeda MA, Vera CA. *Malassezia globosa* as the causative agent of pityriasis versicolor. *Brit J Dermatol* 2000;143:799-803.
15. Aspiroz C, Ara M, Varea M. Isolation of *Malassezia globosa* and *M. sympodialis* from patients with pityriasis versicolor in Spain. *Mycopathologia* 2001;154:111-117.
16. Tarazooie B, Kordbacheh P, Zaini F. Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran. *BMC Dermatol* 2004;4:5.
17. Gupta AK, Kohli Y, Faergemann J, Summerbell RC. Epidemiology of *Malassezia* yeasts associated with pityriasis versicolor in Ontario, Canada. *Med Mycol* 2001;39:199-206.
18. 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.
19. Guillot J, Gue'ho E, Lesourd M. Identification of *Malassezia* species, A practical approach. *J Mycol Med* 1996;6:103-110.
20. Crespo Erchiga V, Ojeda Martos AA, Vera Casaño A, Crespo Erchiga A, Sánchez Fajardo F. Isolation and identification of *Malassezia* spp. In pityriasis versicolor, seborrheic dermatitis and healthy skin. *Rev Iberoam Micol* 1999;16(S):S16-21.
21. Kindo AJ, Sophia SK, Kalyani J, Anandan S. Identification of *Malassezia* species. *Indian J Med Microbiol* 2004; 22(3):179-81.
22. Salah SB, Makni F, Marrakchi S. Identification of *Malassezia* species from Tunisian patients with pityriasis versicolor and normal subjects. *Mycoses*. 2005;48(4):242-5.
23. Crespo-Erchiga V, Florencio VD. *Malassezia* yeasts and pityriasis versicolor. *Curr Opin Infect Dis* 2006;19(2):139-47.
24. Krisanty RI, Bramono K, Made Wisnu I. Identification of *Malassezia* species from pityriasis versicolor in Indonesia and its relationship with clinical characteristics. *Mycoses* 2009;52(3):257-62.
25. Karaka° M, Turaç-Biçer A, Ilkit M, Durdu M, Seydaođlu G. Epidemiology of pityriasis versicolor in Adana, Turkey. *J Dermatol* 2009;36(7):377-82.
26. Tarazooie B, Kordbacheh P, Zaini F, Zomorodian K, Saadat F, Zeraati H, et al. Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran. *BMC Dermatol* 2004;4:1,5.