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. 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. 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.

Malassezia globosa was found to be a species with high level of esterase and lipase enzyme activity probably. These enzyme play important role in pathogenicity. Data regarding antifungal sensitivity pattern vary from species to species and data are very few. In vitro susceptibility
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* species\(^{25}\). 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 2013 to 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 days 3 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).

### Discussion

Malassezia yeasts are lipophilic fungi which are normal flora of the skin and are recovered in 75-98% of healthy individuals.\(^{21}\) This 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 2013\(^{14}\) in central India and Moniri et al 2009\(^{12}\) in Iran reported 59.71% male in and 64.4% respectively. Kalyani et al 2014\(^{19}\) in India reported 41% microscopy positivity and 32% culture positivity and Moniri et al 2009\(^{12}\) in Iran reported 96.6% positivity by microscopy and by culture 92%. Aggarwal et al\(^{13}\) 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 al\(^{14}\) in Channi, India reported *M. globosa* in 48.7% case. *M. furfur* in 34.28%
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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 stray 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