

# DETECTION OF MALASSEZIA SPECIES IN PATIENTS OF PITYRIASIS VERSICOLOR AND SEBORRHEIC DERMATITIS USING NESTED-PCR IN DR. WAHIDIN SUDIROHUSODO GENERAL HOSPITAL AND ITS NETWORK HOSPITAL MAKASSAR IN 2018

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**ABSTRACT Introduction** Malassezia is a lipophilic dimorphic fungus belonging to the normal flora of human skin which might cause superficial mycosis on humans in the form of pityriasis Versicolor and may accompany seborrheic dermatitis. Seborrheic dermatitis may affect adults and infants in the rich in sebaceous glands areas such as the face, chest, back and head area. **Aim** This study aims to describe the species of Malassezia species found in pityriasis Versicolor and seborrheic dermatitis in Dr Wahidin Sudirohusodo general hospital and its network hospitals Makassar in 2018. **Method** A cross-sectional study was performed on 29 patients of pityriasis Versicolor, and 40 patients with seborrheic dermatitis who came for treatment at Dr Wahidin Sudirohusodo general hospital and its network hospital in Makassar by Nested PCR examination of scale specimens scraped from both groups of patients with each primer to identify species of Malassezia species. Frequency distribution test was conducted by Fisher's exact test. **Results** The results showed 29 patients pityriasis Versicolor and 40 patients seborrheic dermatitis. Of the three species of Malassezia (M.globosa, M.furfur, M.restricta), with Nested PCR examination, only 14 cases of M. restricta were found in pityriasis Versicolor patients (48.3%) and 31 cases of seborrheic dermatitis (77.5%). M. furfur and M.globosa were not found in both groups of patients. **Conclusions and Recommendations** M.restricta was found on seborrheic dermatitis, and its prevalence is more significant than its finding on pityriasis Versicolor. Therefore, antifungals should be considered to be given to patients with seborrheic dermatitis.

**KEYWORDS:** Seborrheic Dermatitis; Malassezia; Nested-PCR; Pityriasis Versicolor.

## INTRODUCTION

Malassezia is a normal flora which commonly situated on the skin. Hence in some cases, Malassezia might reproduce in large quantities resulting in disease. Malassezia belongs to lipophilic and or lipid-dependent groups. This yeast habitat is the skin of humans and other warm-blooded animals. Found in 90% of healthy adult skin, this organism can change its state of saprophyte into a pathogen under the influence of predisposing factors, such as changes in skin microflora and or changes in host

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defence.[1]

Malassezia species may cause pityriasis Versicolor and are associated with the pathogenesis of skin disorder such as seborrheic dermatitis, atopic dermatitis, and recent finding shows their involvement in the occurrence of psoriasis. Pityriasis Versicolor was first observed in 1801 by Willan. In 1889, Baillon suggested that the Malassezia genus was the cause of pityriasis Versicolor.[2]

Previous study done in Ahvaz Iran shows that most species involved in pityriasis Versicolor and seborrheic dermatitis were *M. furfur* (51.3%) and *M. restricta* (65.2%), other species such as *M. globosa* (35.2%) and *M. restricta* (13.5%) were found in pityriasis Versicolor, and *M. globosa* (26.1%), *M. furfur* (8.7%) were found in seborrheic dermatitis. We did not find any other species in this study.[1]

Pityriasis Versicolor (PV) is a superficial fungal infection caused by yeast and lipophilic fungi of the Malassezia genus, characterised by fine scaly patches (pityriasis), hypochromic or hyperchromic (Versicolor), and usually present in the neck, body and arms. Its infection might extend to face, groin and thigh.[3]

The study of several experts in various sites on the colonisation of Malassezia species in PV patients showed varying results. This suggests a geographical variation in the prevalence of different Malassezia species in patients with pityriasis Versicolor.[4]

Seborrheic dermatitis (SD) is a chronic skin disorder commonly affecting adults and infants. It is situated in the areas of the body with a high concentration of sebaceous follicles and active sebaceous glands including face, scalp, the upper part of the body, and the body folds (inguinal, inframammary, and axilla). Clinical features of them are pinkish to red plaque and superficial spots with yellowish, and sometimes oily, scales. Seborrheic dermatitis is common in patients with infections of Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) as well as in neurological disorders, such as Parkinson's disease.[5]

PCR (Polymerase Chain Reaction) is an enzymatic method for DNA amplification by way of in vitro. Several significant components such as DNA mould, primary oligonucleotides, deoxyribonucleotide triphosphate (dNTP), DNA polymerase enzymes, and buffering compounds are needed to do PCR. Nested-PCR is a replication technique of DNA samples using DNA polymerase enzymes which have two pairs of primers to amplify the fragments. Using Nested-PCR, any erroneous fragment amplified by the first primer might be amplified a second time by the second primer. Lim et al. (2008), using Nested-PCR to identify Malassezia species in patients with seborrheic dermatitis.[6] Recent studies in Ahvaz, Iran, also identified dominant species in patients with pityriasis Versicolor and seborrheic dermatitis by using this method. The results of the dominant Malassezia species were *M. furfur* followed by *M. restricta*.[1]

This study aimed to describe the species of Malassezia species found in pityriasis Versicolor and seborrheic dermatitis in Dr Wahidin Sudirohusodo general hospital and its network hospitals Makassar in 2018.

## MATERIALS AND METHODS

The study was conducted in the Division of Skin and Genitalia of Dr Wahidin Sudirohusodo general hospital and its network hospitals Makassar in 2018 including clinical determination of pityriasis Versicolor and seborrheic dermatitis. The Nested-PCR examination was performed at the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University.

This research is observational research conducted with cross-sectional approach. The research variables consist of the dependent variable (Malassezia species) and independent variable (pityriasis Versicolor and seborrheic dermatitis).

The population of the study was pityriasis Versicolor and seborrheic dermatitis patients treated in Skin and Genitalia clinic or inpatient of Dr. Wahidin Sudirohusodo general hospital and its network hospital Makassar in 2018. The samples of research are all patients who meet the criteria that are as many as 69 people consisting of 40 people with seborrheic dermatitis and 29 people with pityriasis Versicolor.

The subjects who met the criteria of the study sample were classified into two groups, such as PV and SD. Their skins were then collected by skin scraping after the skin lesions were cleansed with alcohol swabs. It was done by using a blunt scalpel to obtain scale, which was then accommodated on a glass object that has been passed on fire spiritus. The specimen was then inspected by using KOH. Hereafter, DNA of the Malassezia was isolated to be tested with Nested-PCR.

The data in this study was processed by computer statistical software using SPSS version 20. To test the analysis hypothesis, Chi-square test was used. The hypothesis is accepted when the value of  $P < 0.05$  with 95% confidence interval.

## RESULTS

This study has 69 samples consisting of 40 people with seborrheic dermatitis and 29 people with pityriasis Versicolor who meets the inclusion criteria from Dr Wahidin Sudirohusodo general hospital and its network hospitals. The examination was done by taking into account the clinical manifestations to determine the clinical diagnosis. Scale specimens were taken by scraping it from the lesions of seborrheic dermatitis and pityriasis Versicolor patients. Nested-PCR then examined them performed to identify Malassezia species at Microbiology Laboratory of UNHAS Makassar Hospital. This study aims to determine the frequency or incidence of Malassezia species found in patients with seborrheic dermatitis and pityriasis Versicolor.

Characteristics of patients with seborrheic dermatitis and pityriasis Versicolor showed that there was a significant difference in the age of seborrheic dermatitis patients with pityriasis Versicolor ( $p < 0.05$ ). Pityriasis Versicolor patients were found at younger ages ( $28.69 \pm 13.96$  vs  $45.88 \pm 9.32$ ). No differences in sex distribution were found ( $p > 0.05$ ); More men were found in both groups (Table 1).

The findings of Malassezia species in patients with seborrheic dermatitis and pityriasis Versicolor showed that *M. restricta* species were found in both groups, but no *M. furfur* and *M. globosa* species were found in both groups (Table 2).

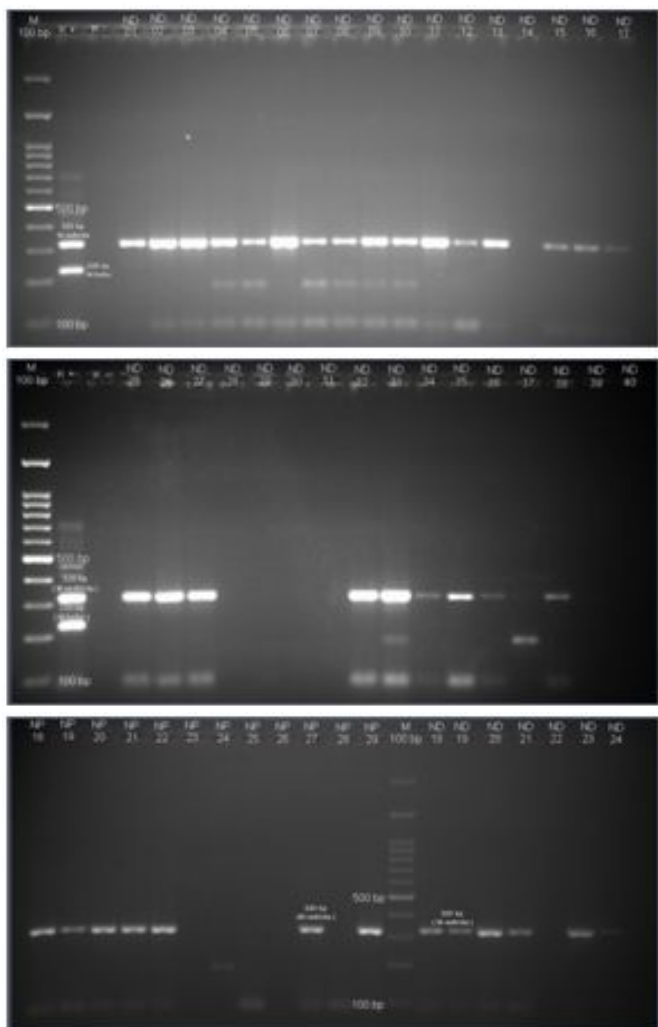
$\chi^2$  test results showed a significant difference of *M. restricta* findings ( $p < 0.05$ ) between patients with seborrheic dermatitis compared to Pityriasis Versicolor. The findings of *M. restricta* in Seborrheic Dermatitis were higher than the findings of *M. restricta* on Pityriasis Versicolor (77.5% vs 48.3%) (Table 3).

Electrophoresis of PCR products in the sample group of patients with seborrheic dermatitis and pityriasis Versicolor showed positive predominance detecting *M. restricta* on target band of 320 bp with CTTGGTTGGACCGTCACTG forward primer and AGCGGATGCAAAGTGTCTC reverse primer. (Figure 1)

**Table 1** Characteristics of patients with seborrheic dermatitis and pityriasis Versicolor

Characteristics		Seborrheic Dermatitis (n=40)	PityriasisVersikolor (n=29)	The Value of p
Age (years)	Min/Max	29/68	4/56	<0,0001
	Mean(SD)	45,88(9,32)	28,69(13,96)	<0,0001
Gender	Male; n (%)	27 (67,5%)	21(72,4%)	0,863
	Female; n (%)	13 (32,5%)	8(27,6%)	0,863

\*independent sample t test; Continuity correction X2 test



**Figure 1:** Nested-PCR examination results

**Table 2** Malassezia species findings in patients with seborrheic dermatitis and pityriasis Versicolor

Malassezia Species	Seborrheic Dermatitis (n=40)		Pityriasis Versicolor (n=29)	
	N	%	n	%
M.furfur	0	0,0%	0	0,0%
M.globosa	0	0,0%	0	0,0%
M.restricta	31	77,5%	14	48,3%

## DISCUSSION

Seborrheic dermatitis is a skin condition with sub-acute or chronic inflammation, characterised by pruritus, oily erythematous plaque, yellowish-grey scales seen in rich in sebaceous glands areas such as the face, head, upper chest, and back. Seborrheic dermatitis accounts for about 3-5% of adult population, with a tendency to occur in men.[5,7]

The cause of seborrheic dermatitis is not yet known, hence some factors which play a role in its etiopathogenesis is the Malassezia species.[8] Recent clinical studies show that increased density of Malassezia has an important role in the pathogenesis of seborrheic dermatitis.[9] Seborrheic dermatitis occurs in adolescence or young adulthood, with increased incidence in patients older than 50 years.8 In elderly patients, the reduced immune system made them more susceptible to various diseases such as seborrheic dermatitis. In Indonesia, retrospective research results from Malak et al. (2016), shows the highest distribution for seborrheic dermatitis incidence was from 45-64 years group in Manado.[10]

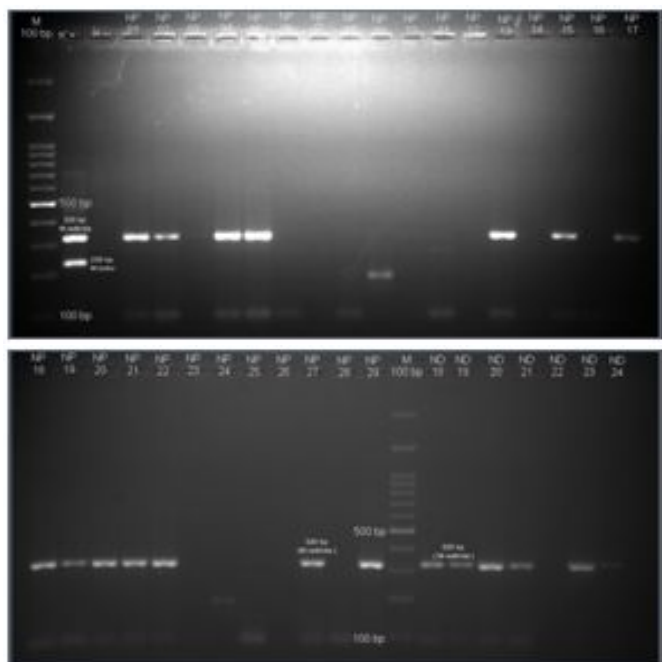
In this study, the distribution of cases of seborrheic dermatitis obtained on men was more than women. The number of men samples were 27 cases (67,5%) whereas women were only 13 cases (32,5%). This is similar to previous research by Malak et al. (2016), in Manado, the 61 cases were men (67.0%), and 30 cases were women (33.0%). It is probably associated with higher androgen hormone stimulation in men than in women where this hormone has a function to produce sebum and enhance sebum activity which is one of the causes of seborrheic dermatitis.[10]

The study found M.restricta species, but no M. furfur and M.globosa species were found in both groups. The species of Malassezia detected in a positive sample of seborrheic dermatitis was M. restricta. According to the literature, most studies

**Table 3** Distinct distribution of findings of *M.restricta* species between seborrheic dermatitis patients and pityriasis Versicolor

Clinical Diagnose	M.restricta				The Value of p
	Positive (n=45)		Negative (n=24)		
Seborrheic Dermatitis	31	77,5%	9	22,5%	0,024
Pityriasis versicolor	14	48,3%	15	51,7%	

\*Continuity Correction X2 test



**Figure 1** (continued)

around the world show *M. restricta* as the dominant species in patients with seborrheic dermatitis. Research conducted by Lim et al (2008), using the Nested-PCR method, and Lee et al (2011), using the PCR-RFLP method to identify *Malassezia* species in patients with seborrheic dermatitis in Korea, obtained the results of dominant species such as *M. restricta* (47%) and *M. furfur* (27%).[6,11] Thus the results of this study are similar to those of previous researchers.

Cases of pityriasis Versicolor also found in more men than women. The number of men was 21 cases (72,4%) whereas women were only 8 cases (27,6%). Archana et al. (2015), in Karnataka India also found PV incidence was more common in men (73%) than in women (27%). Gosh et al., Rao et al., and Krishnan et al. also found the tendency in men is likely to be influenced by outer activity in men.[12]

Based on *Malassezia*'s findings in patients with seborrheic dermatitis and pityriasis Versicolor in this study showed that *M.restricta* species were found in both groups, but no *M. furfur* and *M.globosa* species were found in both groups. In 40 patients with seborrheic dermatitis, 31 cases (77.5%) were positive *M.restricta* while 9 cases (22.5%) were negative. Whereas in 29 patients of pityriasis Versicolor, 14 cases (48,3%) were positive *M.restricta* yet 15 cases (51,7%) were negative. In the previous study conducted by Mahmoudabadi et al. (2014), in Ahvaz Iran, the *Malassezia*'s finding in 30 patients with seborrheic dermati-

sis were 15 cases of *M. restricta* (65.2%), 6 cases of *M. globosa* (26, 1%) and 2 cases of *M. furfur* (8.7%). In pityriasis Versicolor group, *M.furfur* was more common as they had 19 cases (51,3%), 13 cases of *M.globosa* (35,2%) and 5 cases of *M.restricta* (13,5%) from 45 cases.[1] *Malassezia* which is the main cause of PV species seems to differ geographically. *M. globose* has been reported more prevalent in many countries with moderate temperature and Mediterranean climates such as Spain, Japan, Israel, northern India, Iran, Tunisia, Italy and Bosnia-Herzegovina. *M. sympodialis* is more common in subtropical countries such as Argentina, Brazil and South India except in Canada which has a moderate temperature. *M. furfur* has shown a clear dominance in tropical countries such as Indonesia, Thailand, Brazil and Venezuela.[13]

In this study we found 4 cases of pityriasis Versicolor having 10% positive potassium hydroxide (KOH) examination results, which was the discovery of "spaghetti and meatballs", but no DNA of *Malassezia* species was found on the Nested-PCR examination. This might due to the Nested-PCR primer which was specific to *M.furfur*, *M.globosa* and *M. restricta*, thus other species then those might not be detected.

## CONCLUSIONS AND RECOMMENDATIONS

We concluded that in pityriasis Versicolor patients, *Malassezia restricta* was the only species found with the prevalence of 48.3%, whereas *M. furfur* and *M.globosa* were not found. In seborrheic dermatitis patients, the only species found was *Malassezia restricta* with the prevalence of 77.5%, whereas *M. furfur* and *M.globosa* were not found. We also suggest that in the treatment of seborrheic dermatitis, antifungal drugs, especially in recurrent patients should be considered. Further research is needed by extending the type of primer used to identify other types of *Malassezia* with the appropriate number of samples.

### Competing Interests

The authors declare no conflict of interest.

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