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# 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 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. 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).

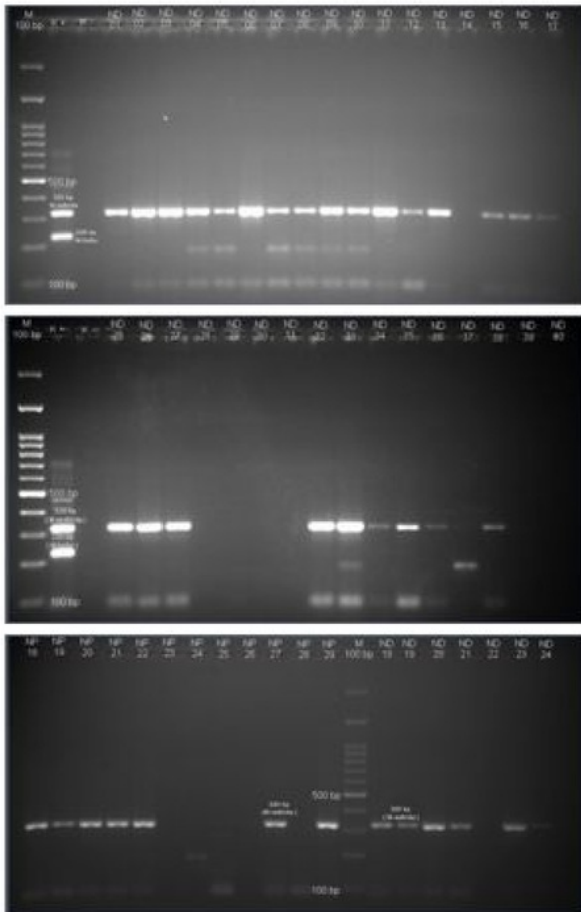
$\chi^2$  test results showed a significant difference of *M. restricta* findings ( $p < 0.05$ ) between patients with seborrheic dermatitis compared to Pityriasis Versicolor. The findings of *M. restricta* in Seborrheic Dermatitis were higher than the findings of *M. restricta* on Pityriasis Versicolor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



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