



Research Paper

Identification of *Malassezia* species isolated from some *Malassezia* associated skin diseasesMervat Hamdino^{a,*}, Amany Ahmed Saudy^a, Laila Hussein El-Shahed^a, Mohamed Taha^b^a Dermatology and Venereology Department, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt^b Microbiology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

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ABSTRACT

Background: The genus *Malassezia* represents the dominant eukaryotic component of the skin microbial flora. There are complex interactions between this commensal and the skin, leading to various *Malassezia*-caused or *Malassezia* exacerbated skin conditions.

Objectives: To identify *Malassezia* species in lesions of patients with pityriasis versicolor (PV), atopic dermatitis (AD), and seborrheic dermatitis (SD), as well as corresponding sites in healthy subjects according to the culture methods used for *Malassezia* species isolation.

Methods: Scrapings were collected from 80 patients (40 PV, 20 AD, and 20 SD) and 30 healthy subjects. For 10–14 days, specimens were cultured on Dixon's medium and Malt extract agar. Direct microscopic examination with Gram's stain, subculture on Hi chrome agar, Dixon's medium at various temperatures, Tweens assimilation, and hydrolysis of tryptophan were used for the identification of yeast isolates.

Results: The isolation frequency of *Malassezia* species in healthy subjects was 13.3% for *M. furfur*, 10.0% for *M. globosa*, and 3.3% for *M. sympodialis*. In patients with SD, *M. furfur* was isolated more frequently from scalp lesions (25.0%) and then *M. sympodialis* (15%) and *M. globosa* (10%). *Malassezia sympodialis* was the most prevalent isolated species in AD lesions (20%), followed by *M. furfur* (10%). *Malassezia* species isolation was found to be most prevalent in PV lesions, with *M. furfur* being the most prevalent identified species (52.5%), followed by unidentified species (20%).

Conclusions: *Malassezia* species composition was similar in PV, SD, and healthy subjects, with *M. furfur* being the commonest isolate, while *Malassezia sympodialis* was the prevalent species isolated in AD lesions. Chrome agar media can be promising for the identification of *Malassezia* species phenotypically. However, species differentiation has to be complemented by molecular methods.

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Introduction

Malassezia are lipid-dependent basidiomycetous yeasts and integral members of the skin microbiome. The *Malassezia* genus currently includes 18 species and numerous functionally distinct strains [1]. It remains controversial whether *Malassezia* are benign commensals or pathogens. It is quite acceptable that differing circumstances can lead to either. The interaction between fungi and the human host is complex and pathogenicity is defined not only by the presence or absence of a specific microbe but also by the complex interplay of the environment and the host susceptibility, which has led to a model of commensalism versus pathogenicity that includes both direct damage from the

microbe and damage induced by the host response, termed 'damage-response framework' [1]. *Malassezia* has the potential to interact with almost all the epidermis cells, such as keratinocytes, melanocytes, Langerhans cells, and the immune system [2]. *Malassezia* spp. are a known cause of pityriasis versicolor (PV) and *Malassezia* folliculitis (MF). While they act as a triggering factor in seborrheic dermatitis (SD) and atopic dermatitis (AD) [3]. Differentiation between *Malassezia* species by phenotypic characteristics may be difficult due to their similar morphological and biochemical features. Molecular methods for *Malassezia* identification are more accurate, but they are not available in most clinical laboratories. Subsequently, culture methods are needed for *Malassezia* species identification [4]. We aimed to isolate and identify *Malassezia* species from patients with pityriasis versicolor, atopic dermatitis, seborrheic dermatitis, and the corresponding sites in healthy subjects according to the culture method used for the isolation of *Malassezia* species.

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Table 1.
Demographic data of the participants

Groups	Number	Sex	Age	Mean $\pm$ SD
PV	40	M 15 F 25	13.00 – 55.00	29.68 $\pm$ 11.51
SD	20	M 5 F 15	3–30	18.90 $\pm$ 7.12
AD	20	M 8 F 12	3–30	11.45 $\pm$ 7.03
Healthy controls	30	M 12 F 18	3–30	17.17 $\pm$ 8.07

PV, pityriasis versicolor; SD, seborrheic dermatitis; AD, atopic dermatitis; F, female; M, male.

Materials and methods

Patients

This cross-sectional descriptive study involved three groups of patients (group I, II, and III, which are PV, SD, and AD, respectively) and group IV (healthy subjects) (Table 1)

Patients in the study and the healthy individuals were the attendants of the Dermatology Outpatient Clinic of Al-Zahraa University Hospital, Cairo, Egypt. We excluded patients using topical antifungals or/and systemic antifungals one month prior to the study. Prior to enrollment, informed written consent was done from all participants after clarifying all the study steps.

Sample collection

The lesions were scraped with a scalpel to obtain specimens. Specimens were taken from the face, neck, upper trunk, and upper limbs of PV patients, the face, scalp, and trunk of SD patients, the face and trunk of AD patients, and the scalp, face, neck, and trunk of healthy subjects. Direct microscopy with KOH 20% was performed on all samples.

Culture

Skin scrapings from each sample were inoculated on two tubes of the following:

- Dixon's medium (HiMedia®, India) (3.6% malt extract, 0.6% peptone, 2% ox-bile, 1% tween 40, 0.2% glycerol and 1.2% agar).
- Malt Extract Agar (HiMedia®, India) (30 gm/l malt extract, mycological peptone 5 gm/l and agar 15gm/l, with addition of olive oil).
- Mycobiotic agar media (Condalab®, Spain) (dextrose 10gm/l, soy peptone 10gm/l, cycloheximide 0.4 gm/l, chloramphenicol 0.05 gm/l and bacteriological agar 15.5 gm/l).

Colonies obtained after incubation for 1–2 weeks with presumptive identified as *Malassezia* yeast by Gram staining were subcultured on HiCrome *Malassezia* agar (Twin pack) (HiMedia®, India) (peptone special 30gm/l and chromogenic mixture agar 15gm/l representing

part A. Part B contained Tween 40 10gm/l, glycerol mono-oleate 5gm/l, and fatty acids 10gm/l).

After incubation for 48–72 hours at 32° C, culture characteristic and color observation were reported.

Tweens assimilation according to the method of Guillot et al. [5]: 0.2 ml of yeast suspension using distilled water was poured into melted Mycosel agar (45 °C). The solidified medium was subjected to 4 wells formation. Then, 40 μ l of Tweens 20, 40, 60, and 80 were added to the wells. The plates were incubated for 7–10 days at 32 °C (Table 2, Fig. 1).

Statistical analysis

Data analysis was performed using the Statistical Package for Social Science, version 23 (IBM SPSS, Armonk, NY, USA, IBM Corp). Quantitative data were represented as mean, standard deviations, and ranges, with parametric distribution and median with inter-quartile range (IQR) for non-parametric distribution. Qualitative variables were expressed as numbers and percentages. The Chi-square test was utilized to compare between groups with qualitative data. P-value <0.05 was considered significant.

Results

The prevalence of *Malassezia* species in healthy controls was 26.7% (8/30). The isolated species were *Malassezia furfur* 13.3% (4/30), followed by *Malassezia globosa* (10%) (3/30), then *Malassezia sympodialis* (3.3%) (1/30). Half of identified species were isolated from the trunk (4/8) (Table 3).

In the SD group, the percentage of positive culture was (50%) (10/20). *Malassezia furfur* was also most prevalent isolated species (5/20) (25%), followed by *Malassezia sympodialis* (3/20) (15%), and then *Malassezia globosa* (2/20) (10%). Eight species were isolated from the scalp, and only two species were isolated from the face, while trunk lesions yielded negative cultures (Table 4).

An increased percentage of negative culture was detected in AD lesions (12/20) (60%). *M. sympodialis* was isolated from the face (20%; 4/20), *M. furfur* was isolated from trunk lesions, and the remaining two isolated species were non-identified. (Table 5).

The percentage of positive culture in the PV lesions was (90%) (36/40). *Malassezia furfur* was the dominant isolated species in PV lesions (21/40) (52.5%), then *M. globosa* (5/40) (12.5%), and *M. sympodialis* (2/40) (5%), while eight isolated species were non-identified (20%). There was no significant difference between lesion sites in terms of culture results and organism type (Table 6).

Discussion

In the present study, three *Malassezia* species (*M. furfur*, *M. globosa*, and *M. sympodialis*) were identified from healthy subjects and PV, SD, and AD lesions according to the culture methods used for the isolation of *Malassezia* species.

Table 2.
Morphological and biochemical characteristics of isolated species

Species	Microscopic Gram's stain	Morphology on Hi chrome agar	Growth on Dixon's in different temperatures			Tween assimilation				Hydrolysis of tryptophan
			32	37	41	20	40	60	80	
<i>M-Furfur</i>	Oval or spherical yeast with broad base bud	Large, pale pink and wrinkled colonies	✓	✓	✓	✓	✓	✓	✓	Positive reaction
<i>M-globosa</i>	Spherical yeast with narrow base bud	Small, purple and smooth colonies	✓	✓		✗	✗	✗	✗	Negative reaction
<i>M-sympodialis</i>	Small ovoid cell with sympodial bud	Large, pale pink and smooth colonies	✓	✓	✓	✗	✓	✓	✓	Negative reaction

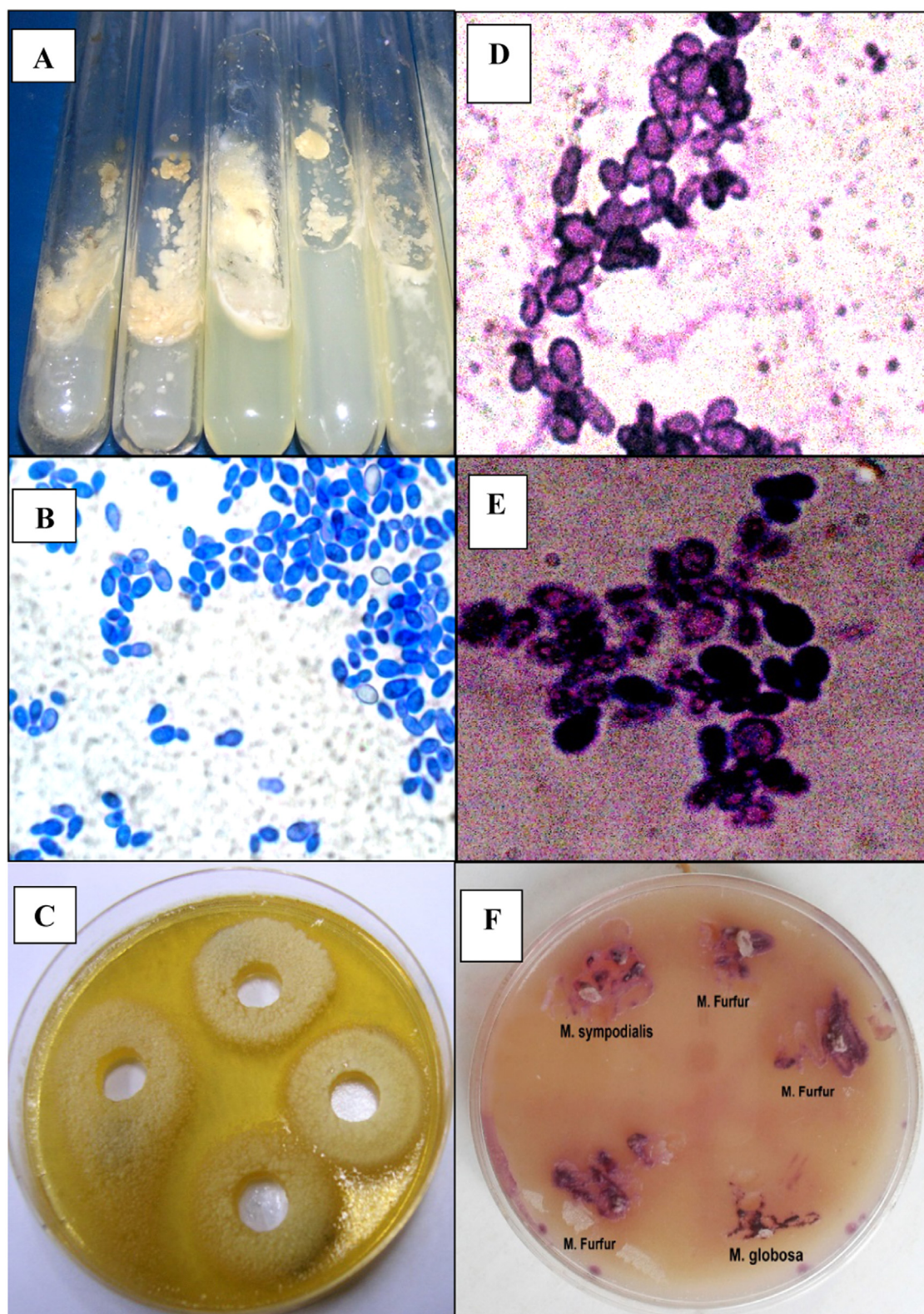


Fig. 1. (A) *Malassezia* species: colonies on Dixon's: tube no. 1,2,5 *M. furfur*, tube no. 3 *M. globosa*, and tube no. 4 *M. sympodialis* (from left). (B) *Malassezia globosa*: spherical yeast cells with narrow base bud after lactophenol cotton blue (LPCB). (C) *Malassezia furfur*: Tweens assimilation test with growth around all wells. T 20 right, T 40 bottom, T 60 left, and T 80 top. (D) *Malassezia furfur*: bottle shaped yeast cells (Gram's stain). (E) *Malassezia sympodialis*: sympodial conidia attached to each other (Gram's stain). (F) Positive culture on Hi chrome agar showing colonies of different *Malassezia* species (*Malassezia furfur*: large wrinkled pale pink colonies, *Malassezia globosa*: Small smooth purple colonies, *Malassezia sympodialis*: Large smooth pale pink colonies).

Table 3.
Isolated *Malassezia* species from healthy subjects.

		Species				Test value	P-value	Sig.
		Negative No. = 22	<i>Malassezia furfur</i> No. = 4	<i>Malassezia sympodialis</i> No. = 1	<i>Malassezia globosa</i> No. = 3			
Site	Face	6 (27.3%)	1 (25.0%)	0 (0.0%)	1 (33.3%)	1.361*	0.968	NS
	Scalp	5 (22.7%)	1 (25.0%)	0 (0.0%)	1 (33.3%)			
	Trunk	11 (50.0%)	2 (50.0%)	1 (100.0%)	1 (33.3%)			
	Neck	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			

* Chi-square test

P-value >0.05: Non-significant (NS)

Table 4.
Isolated *Malassezia* species from seborrheic dermatitis lesions.

		Species				Test value	P-value	Sig.
		Negative No. = 10	<i>Malassezia furfur</i> No. = 5	<i>Malassezia globosa</i> No. = 2	<i>Malassezia sympodialis</i> No. = 3			
Site	Face	3 (30.0%)	1 (20.0%)	1 (50.0%)	0 (0.0%)	3.114*	0.794	NS
	Scalp	6 (60.0%)	4 (80.0%)	1 (50.0%)	3 (100.0%)			
	Trunk	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			

* Chi-square test

P-value >0.05: Non-significant (NS)

Table 5.
Isolated *Malassezia* species from atopic dermatitis lesions.

		Species				Test value	P-value	Sig.
		Negative No. = 12	<i>Malassezia furfur</i> No. = 2	<i>Malassezia species</i> (non-identified) No. = 2	<i>Malassezia sympodialis</i> No. = 4			
Site	Face	8 (66.6%)	0 (0.0%)	1 (50%)	4 (100%)	2.734*	0.435	NS
	Trunk	4 (33.4%)	2 (100%)	1 (50%)	0 (0.0%)			

* Chi-square test

P-value >0.05: Non-significant (NS)

Table 6.
Isolated *Malassezia* species from pityriasis versicolor lesions.

		Pityriasis versicolor group					Test value	P-value	Sig.
		Negative No. = 4	<i>Malassezia furfur</i> No. = 21	<i>Malassezia species</i> (non-identified) No. = 8	<i>Malassezia sympodialis</i> No. = 2	<i>Malassezia globosa</i> No. = 5			
Neck	Negative	4 (100.0%)	11 (52.4%)	7 (87.5%)	1 (50.0%)	4 (80.0%)	1.483*	0.830	NS
	Positive	0 (0.0%)	10 (47.6%)	1 (12.5%)	1 (50.0%)	1 (20.0%)			
Back	Negative	2 (50.0%)	9 (42.9%)	4 (50.0%)	0 (0.0%)	2 (40.0%)	5.863*	0.210	NS
	Positive	2 (50.0%)	12 (57.1%)	4 (50.0%)	2 (100.0%)	3 (60.0%)			
Chest	Negative	2 (50.0%)	13 (61.9%)	5 (62.5%)	2 (100.0%)	3 (60.0%)	4.103*	0.392	NS
	Positive	2 (50.0%)	8 (38.1%)	3 (37.5%)	0 (0.0%)	240.0%)			
Arms	Negative	3 (75.0%)	9 (42.9%)	6 (75.0%)	2 (100.0%)	480.0%)	5.863*	0.210	NS
	Positive	1 (25.0%)	12 (57.1%)	2 (25.0%)	0 (0.0%)	120.0%)			
Abdomen	Negative	4 (100.0%)	21 (100.0%)	7 (87.5%)	2 (100.0%)	5 (100.0%)	4.103*	0.392	NS
	Positive	0 (0.0%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)			
Type	Hyperpigmented	4 (100.0%)	17 (81.0%)	7 (87.5%)	2 (100.0%)	5 (100.0%)	2.395*	0.664	NS
	Hypopigmented	0 (0.0%)	4 (19.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)			

* Chi-square test

P-value >0.05: Non-significant (NS)

Gue'ho et al. [6] demonstrated that *M. globosa* was regularly isolated in PV and SD and that both *M. sympodialis*, with *M. restricta* were commonly found in both healthy and diseased scalp and face skin. In addition, Leeming et al. [7] reported the prevalence of three *Malassezia* species, *M. sympodialis* and *M. globosa*, were dominant on the back, while *M. restricta* was found on the forehead. *M. globosa* and *M. sympodialis* were also frequently detected on the forehead. However, the specific relationships of the *Malassezia* species to such

diseases as AD, SD, and PV are variably affected by geography, seasonal variation, and severity of lesions. Consequently, the relationships between the species of *Malassezia* and these diseases are still under investigation.

The current study results demonstrated *M. furfur* as the most common isolated species in PV, SD, and healthy individuals. Consistent with our findings, Krisanty et al. [8] reported that *Malassezia furfur* was the first causative agent in PV by culture-based methods and

demonstrated the effect of geography on the predominant species from PV lesions. According to reports, *Malassezia furfur* is the dominant species in tropical climate and *Malassezia globosa* prevails in temperate regions. Also, Diongue et al. [9] found that only *M. furfur* was identified in 100% of PV in Dakar.

Nkabayashi et al. [10] reported the isolation of 35% *M. furfur* and 22% *M. globosa* from lesional SD in Japanese patients and *M. globosa* as the dominant species of PV lesions.

Nkabayashi et al. [10] followed Guillot et al. [5] method, which depends on species differences in morphological features, lipid dependency, and Tweens assimilation. Similarly, Hi chrome *Malassezia* agar and Dixon's medium, Tweens assimilation, and tryptophan analysis were used for species identification in the current study. This method has the advantages of simplicity, convenience, and low cost. However, it has some limitations, such as difficulty identifying mixed species. Nkabayashi et al. [10] reported that *M. restricta* was isolated at a very low rate.

In the present study, eight species from PV lesions and two from AD lesions were non-identified, indicating that some species may have been mixed in the primary cultures. In line with these findings, Nkabayashi et al. illustrated that the strain was labeled as 'unknown' when the Tween test did not correspond to any pattern. Therefore, different methods of identification for differentiating species mixtures must be established.

However, phenotypic methods are relatively time-consuming and cannot differentiate the newly identified spp. Molecular methods are accurate and faster for identifying *Malassezia* yeasts as they have higher sensitivity and specificity [11]. Molecular methods have been established to perform faster and more precise identification of *Malassezia* spp. as compared to phenotypic methods, especially for species non-identified by phenotypic methods [12].

Tajima et al., [13], using a PCR-based, culture-independent method, illustrated that the major *Malassezia* species in SD were *M. globosa* and *M. restricta*, found in 93, and 74% of the patients, respectively, and AD showed higher values. Real-time PCR assay demonstrated that the lesional skin harbored nearly three times the population of genus *Malassezia* found in nonlesional skin, and *M. restricta* is a significantly more common species in SD than *M. globosa*.

In contrast to our findings, Gaitanis et al. [14] reported that traditional identification methods lack enough variables to identify different species.

Our study demonstrated that *M. sympodialis* was the most prevalent isolate in AD lesions (20%), followed by *Malassezia furfur* (10%). Patients with negative culture were (60%).

The false results can be attributed to the inadequate sampling area, too many or too few scales in the samples, prior antifungal treatment, or inexperienced interpretation.

Consistent with our results, Gupta et al. [15] reported *Malassezia sympodialis* as a predominant isolate in atopic dermatitis.

Also, Yim et al. [16] demonstrated *M. sympodialis* as a predominant species in Korean atopic dermatitis using molecular techniques.

Our results showed no statistically significant relation between isolated *M. species* and age, sex, site of lesion, type of pigmentation in PV, and severity in SD and AD. The small sample size is a limitation of the present study. A large cohort is required to find a specific relation with these clinical parameters.

In accordance, previous studies also revealed no significant correlation between isolated species and clinical parameters [8, 15, 16].

According to the evidence previously demonstrated, the differences among the studies may be the result of ethnic and geographical variation in species prevalence, different sampling techniques (scraping/contact plates), and different culture media (modified Dixon agar/Leeming-Notman agar) [17].

The finding of *M. furfur* predominance in the tropics might be explained by the recovery of pityriacitrin, an indole alkaloid

produced by *M. furfur*. Pityriacitrin can protect against ultraviolet exposure, which renders *M. furfur* more resistant to sun exposure [18].

Therefore, the distribution of *Malassezia* species on various sites is proportional to the density and activity of pilosebaceous glands in these areas. The higher ambient temperature in our area most likely promotes the spread of lesions to multiple body sites, depending on species dominance.

A better understanding of the distribution of *Malassezia* species serves to complement previously conducted studies of similar topics in various countries and aids in determining trends in species distribution based on climate differences. Moreover, identifying the dominant species for a particular country and their correlation to specific clinical characteristics can lead to a better tailored clinical approach to treating PV, SD, and AD lesions for patients of that locality.

Conclusion

Our study, which aimed to identify *Malassezia species* in patient with PV, SD, AD, and healthy subjects by the culture-based methods, demonstrated that *M. furfur* was the most dominant species in PV, SD and healthy subjects while, *Malassezia sympodialis* was most prevalent from AD lesions. Chrome agar media can be promising for identification of *Malassezia species* phenotypically. Further research is needed to compare lesional and non lesional samples and compare Chrom agar media with molecular techniques to identify different *Malassezia species* in a larger cohort.

Declaration of Competing Interest

The authors have no conflict of interest to declare

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None received

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