Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Morphological Study of *Malassezia* Species Isolated from Pityriasis Versicolor Patients in Thi-Qar Governorate and Their Sensitivity to Some Antifungal Drugs

Marwa Amer Jalil[1], Mohammed Hashim Yasir[2]

[1]Department of Biology, College of Education for women, AL-Shatra University, Iraq.
[2]Department of Biology, College of Science, University of Thi-Qar, Iraq.
Corresponding author: Marwa Amer Jalil
Email: marwa.ameer@utq.edu.iq

Abstract:

Background: *Malassezia* are lipophilic yeasts that coexist symbiotically on the skin of humans and other warm-blooded animals. They assume two forms: unicellular yeasts and pseudofilamentous yeasts. *Malassezia* yeasts are opportunistic and exhibit coexistence with their host without adverse effects. However, they are capable of causing infection in humans under specific circumstances that facilitate their growth, such as a compromised immune system and a pH change. Approximately 80% of healthy individuals harbor *Malassezia* yeasts, which naturally colonize the epidermis of humans around three months after birth.

The Pityriasis versicolor (PV) is induced by *Malassezia* yeast. The yeasts invade the outermost keratinized layer of human skin and induce several alterations in this layer, such as the formation of different-colored patches on the skin. The spots are scaly and can be classified into two types: hypopigmentation, which refers to a condition where the spots are lighter than the skin tone, and hyperpigmentation, which characterizes a condition where the spots are darker than the skin color, resulting in red spots. This condition manifests on many regions of the skin, encompassing the arms, upper body, neck, and face. Furthermore, it manifests on the hair, and it is rarely observed to impact the nails. This infection may be asymptomatic or it is accompanied by mild pruritus.

Objective: This study aims to isolate and phenotypically identify *Malassezia* species from pityriasis versicolor patients, as well as compare their prevalence in patients with that of healthy individuals.

Methods: About 72 skin scraping samples were collected from patients diagnosed by the dermatologist as pityriasis versicolor patients and 30 skin swabs from healthy individuals at the period from Jul-2023 to Dec-2023. All samples were tested for morphological identification and anti-fungal sensitivity of *Malassezia* Spp.

Results: The study revealed that the prevalence of *Malassezia* species infection was higher among patients aged 31-40 years (40% in 24 patients) and 21-30 years (35% in 21 patients). The chest region had the greatest incidence of infection (37.33% in 23 patients) and neck (30.00% in 18 patients). Observation of clinical data verified the existence of hyperpigmented lesions in 40 patients and hypopigmented lesions in 20 patients. All isolates of *Malassezia* shown significant resistance to ketoconazole, fluconazole, clotrimazole, amphotericin, and itraconazole treatments. Notably, ten isolates exhibited the greatest sensitivity to nystatin.

Conclusion: The prevalence of *Malassezia* species infection is higher in males compared to females, and it is quite uncommon in children. They either induce direct disease or actively contribute to the progression of pre-existing skin disorders.

Keywords: *Malassezia* species, Pityriasis versicolor, Antifungal drugs.

Introduction: The primary cause of pityriasis versicolor is the species of yeasts from the genus *Malassezia*, which were previously known as *Pityrosporum*. They are opportunistic yeasts that exist as symbionts or normal flora on human epidermis and are lipophilic, dimorphic, and unipolar budding yeasts. When the appropriate conditions are met, they transition from their yeast form to the pathogenic hyphal form, which can result in a systemic or cutaneous infection (1). *Malassezia* yeasts are classified within the Phylum Basidiomycota and comprise approximately 17 species (2). The presence of *Malassezia* in symbiosis on human skin is neutral or beneficial, as it contributes to the healthy development of a healthy barrier and the balance of skin immune cells throughout life (3). Besides pityriasis versicolor, *Malassezia* is implicated in the development of other diseases, such as seborrheic dermatitis, dandruff, atopic dermatitis, pilonidal folliculitis, seborrheic blepharitis, psoriasis, steroid acne, papillomatosis, and reticulate (4).

Pityriasis versicolor (PV) is a chronic, recurrent superficial fungal infection that is caused by yeasts of the *Malassezia* genus. It manifests in the form of numerous spots on various body parts, such as the face, neck, chest, back, and upper arm, and it impacts both males and females. The infection manifests as a multicolored skin rash that is either lighter in color than the skin, darker in color than the skin, or appears scarlet. The spots are irregular in shape and have a smooth, scaly appearance (5). Pityriasis versicolor is prevalent in all regions of the world, particularly in regions with hot and humid climates. It affects both males and females, but it is more prevalent among youthful people. The infection is not contagious; however, it is more likely to spread among individuals who have a genetic predisposition, hyperhidrosis, poor general health, and an immune system that is compromised (6). The majority of *Malassezia* species are distinguished by their reliance on fatty acids for growth. This is due to their inability to synthesize long-chain saturated fatty acids, which results in a lack of cellular fatty acids. Consequently, they require fatty acids from external sources (7). These yeasts colonies areas of the body that are rich in sebaceous glands, where they colonies the skin of healthy adults more than the skin of infants or pre-pubertal ages, as they are lipophilic and require external fat sources to survive (8). The pathogenesis of these yeasts is characterized by the production of several

critical enzymes that facilitate the growth of the yeasts and induce inflammation, irritation, and crusting in the affected area. These enzymes include lipases, proteases, esterases, phospholipases, and lipoxygenases (4).

Aim of the Study: The current study aims to isolate and morphologically identify *Malassezia* species from Pityriasis versicolor patients in northeastern and central Thi- Qar Governorate, while also estimating their sensitivity to various antifungal drugs.

Methods:

Subjects: From July 15, 2023, to December 30, 2023, 72 skin scraping samples were taken from people with pityriasis versicolor in dermatology consultation clinics in the northeastern and central Thi-Qar governorate. These clinics were AL-Nasiyriah, AL-Shatra, AL-Graph, AL-Nasser, AL-Rifaei, Qaleat Sukar, and AL-Aslah. Additionally, as a control, we collected 30 skin swap samples from healthy individuals of both sexes. We followed an information form to register each patient's details, including gender, age, site of infection, type of infection, and accommodation.

Sample Collection: The PV samples were obtained by scraping the skin with a sterile operating blade and subsequently transferring them to sterile plastic containers to guarantee that they remained highly hydrated. Using a cotton swab that contained a culture medium (9), we obtained samples from healthy individuals.

Morphological Study: In order to verify the clinical diagnosis of infections, samples were subjected to direct microscopic examination with 10% potassium hydroxide (10). Furthermore, the samples were cultivated on sabouraud dextrose agar, which was supplemented with penicillin and gentamicin, and either with or without olive oil, to optimize *Malassezia's* growth requirements (11). Also, the dishes were incubated at a temperature of 30 °C for 5–10 days (12). Lactophenol cotton staining was employed to observe the yeast colonies under a microscope (13). Additionally, gram stain (14) is employed to demonstrate the morphological characteristics. In order to identify *Malassezia* species, biochemical tests were implemented, including the catalase test, hemolysis test, urease test, and Polysorbate (tween) assimilation test (tween 20 and 80) (7,14,18,22,23).

Antifungal susceptibility: The test was conducted using a kit (Hexa Antimyco-01 / HX104-1PK) from HiMedia/India. The kit contains six types of antifungals that belong to two groups of antibiotics: the azoles group, which includes ketoconazole, fluconazole, clotrimazole, and itraconazole, and the macrolide group, which includes amphotericin B and nystatin, table 1.

Table (1): Antifungal drugs that used to find out the resistance and sensitive isolates among *Malassezia* species.

Name Of Kit	Antifungal Drugs	Symbol	oncentration N/Mg
Antimyco-01 / HX104-1PK	Ketoconazole	KT	10
	Fluconazole	FLC	25
	Itraconazole	IT	10
	Clotrimazole	CC	10
	Amphotericin B	AP	100
	Nystatin	NS	100

Ethical Approval: The Iraqi Ministry of Health, Thi- Qar Health Directorate, granted approval for the investigation under the document number [189/2023, 19/9/2023]. Additionally, all participants were aware of the study's objectives and provided verbal consent.

Statistical Analysis: The data was exported into an Excel spreadsheet, and the results were analyzed using the analysis tools available in the Excel software to display the mean and standard deviation.

Results: A total of 60 samples of patients were positive for *Malassezia* infections. Positive samples of patients were involved 36 males and 24 females, mean age 32.23 ± 8.43 years (Range: 14-50 years, median: 32 years), table 2.

Table (2): Distribution of study groups based on age and gender.

Gender		Number	Age Range	Mean ± S. D	Median
Patients	Males	36	14-45	31.83 ± 7.94	32
	Female	24	18-50	32.83 ± 9.25	31.5
Control	Males	22	20-50	30.77 ± 8.24	31
	Females	8	21-45	28.5 ± 7.13	27.5

The highest number of patients was found within age group (31-40 years) in 24 patients followed by (21-30 years) in 21 patients, table 3.

Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Table (3): Distribution of study groups based on age groups.

Age Group	Patients			Control			T-Test (P-Value)
	No.	%	Mean ± S. D	No.	%	Mean ± S. D	
14-20	6	10	18.6 ± 1.14	1	3.33	_	0.006
21-30	21	35	26.4 ± 2.35	17	56.67	25.12 ± 3.22	0.29
31-40	24	40	35.58 ± 3.17	9	30	35.67 ± 3.024	0.95
41-50	9	15	45.29 ± 3.03	3	10	45.67 ± 4.04	0.87

Clinical observation confirmed the presence of hyperpigmented lesions in 40 patients and hypopigmented lesions in 20 patients. Table 4 shows the infection sites on the patients' bodies.

Table (4): PV infections according to the patient's body site.

No.	Infection Site	No. Of Isolates	Percentage %
1	Chest	23	38.33
2	Neck	18	30.00
3	Back	12	20.00
4	Shoulder	3	5.00
5	Arm	2	3.33
6	Face	2	3.33
Total		60	100

AL-NAASIRIA and AL-SHATRA District showed the highest rate of infection with *Malassezia* at 28.33%, followed by AL-GRAPH District 16.67%, AL-NASSER District 13.33%, AL-RIFAEI 8.33%, QALEAT SUKAR 3.33%, and AL-ASLAH 1.67%, table 5.

Table (5): PV infections according to geographical regions.

No.	Geographical Regions	No. Of Isolates	Percentage %
1	AL-Nasiriyah	17	28.33%
2	AL-Shatra	17	28.33%
3	AL-Graph	10	16.67%
4	AL-Nasser	8	13.33%
5	AL-Rifaei	5	8.33%
6	Qaleat Sukar	2	3.33%
7	AL-Aslah	1	1.67%

The direct microscopic diagnosis using potassium hydroxide 10% revealed that *Malassezia* spp. appeared in the form of short, wide threads with sporules, giving them the appearance of spaghetti with meatballs, figure 1.

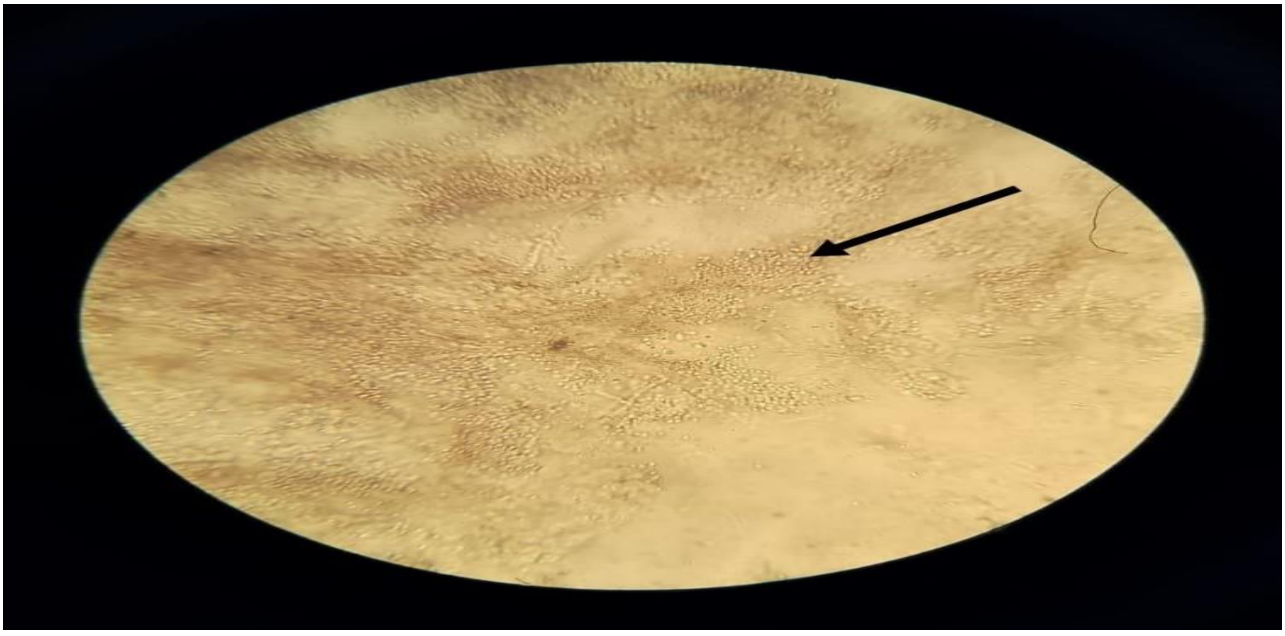

Figure (1): Microscopic Direct examination with KOH

Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

All samples showed growth when cultured on olive oil-containing Modified sabouraud dextrose agar (mSDA) mediums, but not on Sabouraud dextrose agar SDA medium, which does not contain olive oil, figure 2.

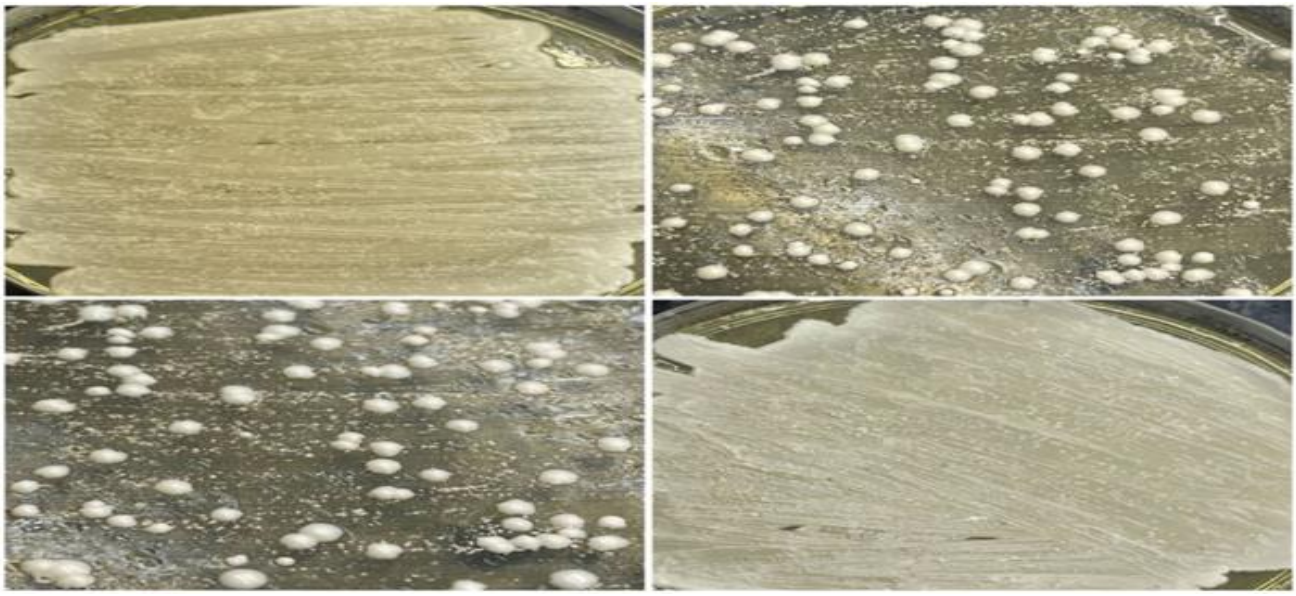

Figure (2). *Malassezia* spp. colonies on mSDA medium

The microscopic diagnosis using lactophenol blue dye revealed that the *Malassezia* cells appeared in a circular or oval shape with a unipolar bud. When using gram stain, *Malassezia* cells were gram-positive and appeared purple under the microscope, figure 3.

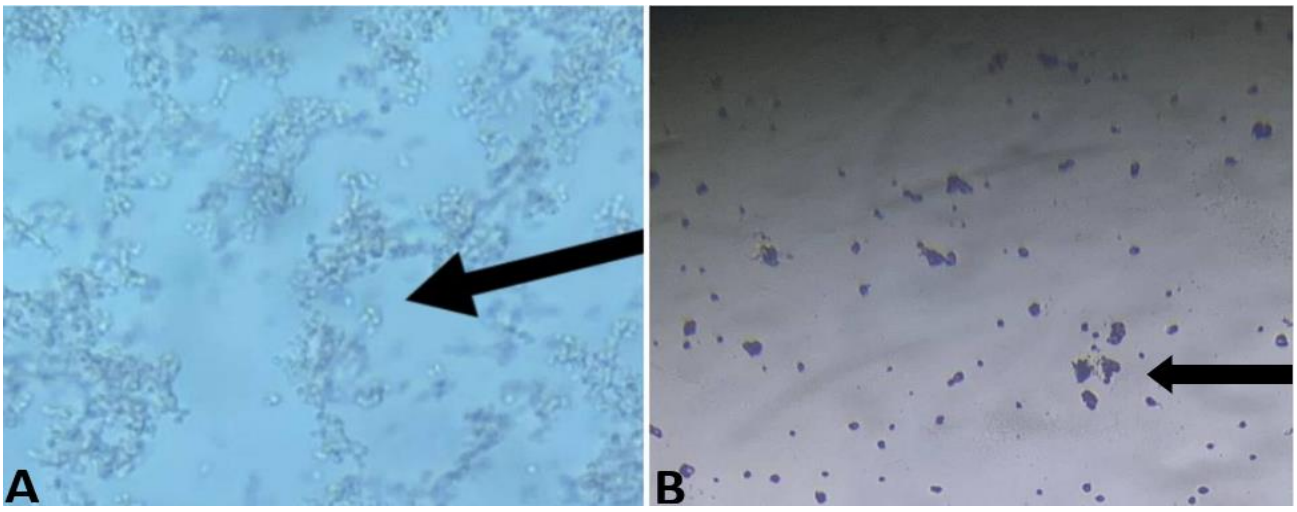

Figure (3): Microscopic appearance of *Malassezia* spp. (A) with Lactophenol cotton blue stain (B) with gram stain.

Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

All *Malassezia* isolates had positive biochemical tests, including the urease test, catalase test, hemolysis test, and growth at 37°C. However, on Tween 20 and 80, not all *Malassezia* species showed growth. *Malassezia furfur* isolates showed positive culture on Tween 20 and 80, but *Malassezia globosa* did not show positive culture in that test, table 6.

Table (6): Biochemical tests of *Malassezia* species.

Test Species	Urease Test	Catalase Test	Hemolysis Test	Tween 20	Tween 80	Growth At 37°c	Growth On SDA	Growth On Msda
Malassezia Furfur	+	+	+	+	+	+	-	+
Malassezia Globosa	+	+	+	-	-	+	-	+

(+) Positive, (-) Negative.

All *Malassezia* isolates were resistant to Ketoconazole and Fluconazole. One isolate was sensitive to clotrimazole and another one was also sensitive to Amphotericin B. Other two isolates were sensitive to Itraconazole. Finally, the highest sensitivity by Nystatin was observed in ten isolates, figure 4.

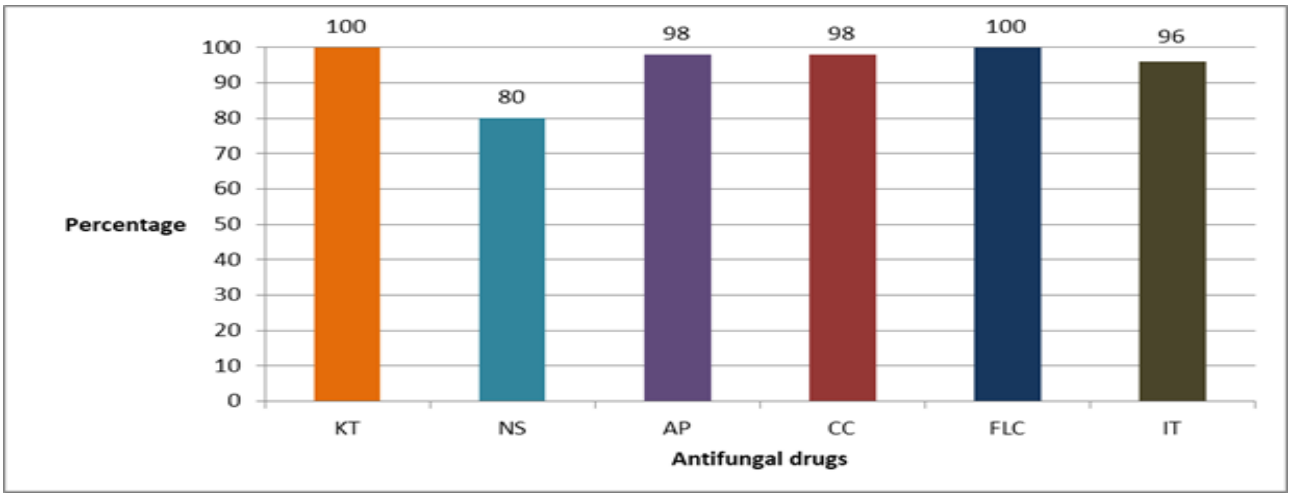

Figure (4): Percentage of antifungal drugs resistance by *Malassezia* isolates according to type of antifungal drug.

Discussion: The current results indicate that the PV infection is more common in males than females in patients' group. This could be attributed to the physiological differences between the sexes or to the nature of Iraqi men, who often engage in hard work that leads to excessive sweating. Men are more likely than women to smoke, which could potentially weaken their immune systems. In general, immune diseases are considered a basic precursor to the occurrence of fungal infection,

including *Malassezia* infection (15). The prevalence of latent *Malassezia* in men was found to be greater than in women in control group. This confirms the reason why the disease appears more in men than in women (16). The age group of 31- 40 years exhibited the highest rate of *Malassezia* infection, with the age group of 21–30 years following closely behind in patients' group. Hormonal changes and high activity of the sebaceous and sweat glands, due to their lipophilic nature, stimulate the growth of *Malassezia* species (9). The Malassezia species isolates from healthy control revealed their prevalence in the age groups of 21-30 years and 31-40 years, mirroring the findings in patients' group (10).

Regarding the site of infection, the results indicated that the chest area was more vulnerable to *Malassezia* infection in the patients' group due to its abundance of sebaceous and sweat glands, as well as the fact that it is frequently covered by clothing. These factors create the ideal conditions for the growth of *Malassezia* (17). In the control group, the prevalence of *Malassezia* in chest area can be attributed to several factors. Firstly, the humidity in this area contributes to the accumulation of oily and sweat secretions, creating a moist environment that fosters the growth of *Malassezia* yeasts. Secondly, the frequent covering of this area with clothing leads to the retention of sweat and fatty secretions, thereby promoting the growth of *Malassezia* species (17).

According to the color of the lesion, the results showed that hyperpigmentation is higher than hypopigmentation. Hyperpigmentation is caused by the thickening and expansion of melanin cells or an immune response in the affected area (18). In the case of hypopigmentation, it could be caused by compounds secreted by *Malassezia* species, such as carboxylic acid or azelaic acid, which inhibit the tyrosine enzyme necessary for melanin pigment synthesis (19).

The prevalence of *Malassezia* in the areas and districts of Thi-Qar Governorate can be attributed to the hot and humid climate in the summer (20). Heat and humidity are one of the factors that trigger the growth of *Malassezia* and its transition from its commensal form to its pathological form (16).

When it's come to the biochemical tests, there were differences between *Malassezia* species, *M. furfur* and *M. globosa*. Both types were positive for the catalase test, hemolysis test, urease test, growth in medium with olive oil, and growth at 37° C (15). While both species were negative for growth without olive oil, *M. globosa* is negative to tween 20 and 80 and *M. furfur* is positive to tween 20 and 80 (21). The results of the antifungal susceptibility test for *Malassezia* species showed that *Malassezia* species exhibited high resistance against most of the antifungals used in this study, especially the azoles group. Many things make *Malassezia* species very resistant to the antifungals used in this study. One reason is the genetic mutations that changes their genes in ways that make them resistant to the effects of antifungals (22). *Malassezia* species also produce enzymes that hydrolyze their antifungal drugs, and these enzymes work to destroy the antifungals before they can the process of physiological adaptation by *Malassezia* species, which alters their cellular structure or metabolic processes to lessen the effect of antifungals, is another factor contributing to their resistance to antifungals (23). Furthermore, the biofilms that *Malassezia* species produce are a significant component of their resistance to antifungals. This is due to the fact that antifungals encounter

significant challenges in navigating biofilms in order to impact these species (24). Finaly, the changes that occur in human skin are another factor that contributes to *Malassezia* yeasts' resistance to antifungals. This means that any changes in the skin environment, such as variations in the skin's pH, immune responses, or climatic conditions such as humidity, all alter *Malassezia's* response to medications (25).

Conclusions: *Malassezia* species tend to infect males more frequently than females, and they rarely infect children. They either cause direct disease or contribute to the development of pre-existing skin diseases. Most *Malassezia* infections were in the chest area. Both *Malassezia* species had negative growth on SDA medium but positive growth on mSDA medium. On Tween 20 and 80, *Malassezia furfur* isolates showed a positive culture, but *Malassezia globosa* did not. *Malassezia* species isolates showed the strongest resistance to ketoconazole and fluconazole, as well as the highest sensitivity to Nystatin.

References:

1. El-Shahed, Laila Hussein, and Mohamed Taha. 2022. "Identification of Malassezia Species Isolated from Some Malassezia Associated Skin Diseases." Journal De Mycologie Medicale 32(4): 101301. doi:10.1016/j.mycmed.2022.101301. https://doi.org/10.1016/j.mycmed.2022.101301.

2. Guillot, Jacques, and Ross Bond. 2020. "Malassezia Yeasts in Veterinary Dermatology: An Updated Overview." Frontiers in Cellular and Infection Microbiology 10: 79. doi:10.3389/fcimb.2020.00079.

3. Swaney, Mary Hannah, and Lindsay R. Kalan. 2021. "Living in Your Skin: Microbes, Molecules, and Mechanisms" ed. Anthony R. Richardson. Infection and Immunity 89(4): e00695-20. doi:10.1128/IAI.00695-20.

4. Abdillah, Abdourahim, and Stéphane Ranque. 2021. "Chronic Diseases Associated with Malassezia Yeast." Journal of Fungi (Basel, Switzerland) 7(10): 855. doi:10.3390/jof7100855.

5. Saunte, Ditte M. L., George Gaitanis, and Roderick James Hay. 2020. "Malassezia-Associated Skin Diseases, the Use of Diagnostics and Treatment." Frontiers in Cellular and Infection Microbiology 10: 112. doi:10.3389/fcimb.2020.00112.

6. Harada, Kazutoshi, Mami Saito, Takashi Sugita, and Ryoji Tsuboi. 2015. "Malassezia Species and Their Associated Skin Diseases." The Journal of Dermatology 42(3): 250–57. doi:10.1111/1346-8138.12700.

7. Hamdino, Mervat, Amany Ahmed Saudy, Laila Hussein El-Shahed, and Mohamed Taha. 2022. "Identification of Malassezia Species Isolated from Some Malassezia Associated Skin Diseases." Journal De Mycologie Medicale 32(4): 101301. doi:10.1016/j.mycmed.2022.101301.

8. Theelen, Bart, Claudia Cafarchia, Georgios Gaitanis, Ioannis Dimitrios Bassukas, Teun Boekhout, and Thomas L. Dawson Jr. 2018. "Malassezia Ecology, Pathophysiology, and Treatment." Medical mycology 56(suppl_1): S10–25. https://doi.org/10.1093/mmy/myx134.

9. Gholami, Mahnaz, Fatemeh Mokhtari, and Rasoul Mohammadi. 2020. "Identification of Malassezia Species Using Direct PCR-Sequencing on Clinical Samples from Patients with Pityriasis Versicolor and Seborrheic Dermatitis." Current Medical Mycology. doi:10.18502/cmm.6.3.3984.

10. Ammari, Abbas M, Saife D Al-Ahmer, and Azhar Al Attraqhchi.2019. "Molecular Study of Malassezia furfur Isolated from Pityriasis Versicolor Patients." https://doi.org/10.32007/jfacmedbagdad.581207.

11. Liu, Xiaoping, Qing Cai, Hong Yang, Zhiqin Gao, and Lianjuan Yang. 2021. "Distribution of Malassezia Species on the Skin of Patients with Psoriasis." Journal De Mycologie Medicale 31(2): 101111. doi:10.1016/j.mycmed.2021.101111.

12. Krzyściak, Paweł, Zofia Bakuła, Agnieszka Gniadek, Aleksander Garlicki, Mikołaj Tarnowski, Michał Wichowski, and Tomasz Jagielski. 2020. "Prevalence of Malassezia Species on the Skin of HIV-Seropositive Patients." Scientific Reports 10(1): 17779. doi:10.1038/s41598-020-74133-6.

13. Chaudhary, Rahul, Sanjay Singh, Tuhina Banerjee, and Ragini Tilak. 2010. "Prevalence of Different Malassezia Species in Pityriasis Versicolor in Central India." Indian Journal of Dermatology, Venereology and Leprology 76(2): 159–64. doi:10.4103/0378-6323.60566. doi: 10.4103/0378-6323.60566.

14. Gyure, Ruth A. 2010. "An Eco-Friendly, Scaled-down Gram Stain Protocol." Journal of Microbiology & Biology Education : JMBE 11(1): 60–61. doi:10.787/jmbe.v1.i2.144.

15. Noor, Abeer T., and Ali A. Alsudani. 2021. "Detection Of Some Virulence Enzymes Of Malasseziaspp. Isolated From Patients Of Pityriasis Versicolor And Their Sensitivity To Some Antifungal Agents." Al-Qadisiyah Journal of Pure Science 26(1): 10–21. doi:10.29350/qjps.2021.26.1.1239.

16. Prohic, Asja, Tamara Jovovic Sadikovic, Mersiha Krupalija-Fazlic, and Suada Kuskunovic-Vlahovljak. 2016. "Malassezia Species in Healthy Skin and in Dermatological Conditions." International Journal of Dermatology 55(5): 494–504. doi:10.1111/ijd.13116.

17. Romano, Clara, Francesca Mancianti, Simona Nardoni, Gaetano Ariti, Paola Caposciutti, and Michele Fimiani. 2013. "Identification of Malassezia Species Isolated from Patients with Extensive Forms of Pityriasis Versicolor in Siena, Italy." Revista Iberoamericana de Micología 30(4): 231–34. doi:10.1016/j.riam.2013.02.001.

18. Jagielski, Tomasz, Elżbieta Rup, Aleksandra Ziółkowska, Katarzyna Roeske, Anna B Macura, and Jacek Bielecki. 2014. "Distribution of Malassezia Species on the Skin of Patients with Atopic

Dermatitis, Psoriasis, and Healthy Volunteers Assessed by Conventional and Molecular Identification Methods." BMC Dermatology 14(1): 3. doi:10.1186/1471-5945-14-3.

19. Archana, Banur Raju, Paravangada Madappa Beena, and Shiva Kumar. 2015. "Study of the Distribution of Malassezia Species in Patients with Pityriasis Versicolor in Kolar Region, Karnataka." Indian Journal of Dermatology 60(3): 321. doi:10.4103/0019-5154.156436.

20. Chebil, Wissal, Najoua Haouas, Raja Chaâbane-Banaoues, Latifa Remadi, Najla Chargui, Selim M'rad, Sameh Belgacem, et al. 2022. "Epidemiology of Pityriasis Versicolor in Tunisia: Clinical Features and Characterization of Malassezia Species." Journal De Mycologie Medicale 32(2): 101246. doi:10.1016/j.mycmed.2022.101246.

21. Awad, Ahmed Kamil, Ali Ibrahim Ali Al-Ezzy, and Ghassan H. Jameel. 2019. "Phenotypic Identification and Molecular Characterization of Malassezia Spp. Isolated from Pityriasis Versicolor Patients with Special Emphasis to Risk Factors in Diyala Province, Iraq." Open Access Macedonian Journal of Medical Sciences 7(5): 707–14. doi:10.3889/oamjms.2019.128.

22. Chebil, Wissal, Wafa Rhimi, Najoua Haouas, Valentina Romano, Sameh Belgacem, Hichem Belhadj Ali, Hamouda Babba, and Claudia Cafarchia. 2022. "Virulence Factors of Malassezia Strains Isolated from Pityriasis Versicolor Patients and Healthy Individuals." Medical Mycology 60(8): myac060. https://doi.org/10.1093/mmy/myac060.

23. Hadrich, Inès, Nahed Khemekhem, Sourour Neji, Houaida Trablesi, Amin Ilahi, Hayet Sellami, Fattouma Makni, and Ali Ayadi. 2022. "Production and Quantification of Virulence Factors in Malassezia Species." Polish Journal of Microbiology 71(4): 529–38. doi:10.33073/pjm-2022-047.

24. Angiolella, Letizia, Claudia Leone, Florencia Rojas, Javier Mussin, María de los Angeles Sosa, and Gustavo Giusiano. 2018. "Biofilm, Adherence, and Hydrophobicity as Virulence Factors in Malassezia Furfur." Medical mycology 56(1): 110–16. https://doi.org/10.1093/mmy/myx014.

25. Grice, Elizabeth A., and Thomas L. Dawson. 2017. "Host–Microbe Interactions: Malassezia and Human Skin." Current opinion in microbiology 40: 81–87. https://doi.org/10.1016/j.mib.2017.10.024.