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Prevalence and Antifungal Susceptibility Profile of *Malassezia* Species Isolated from Pityriasis Patients in Duhok City/ Iraq

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ABSTRACT

Pityriasis versicolor (PV) is a mild, long-lasting superficial cutaneous mycosis caused by the yeast *Malassezia*. It is characterized by distinct or confluent, scaly, discolored spots on the upper trunk and other sebaceous areas. This study aimed to determine the prevalence and antifungal susceptibility profile of *Malassezia* species isolated from pityriasis versicolor patients in Duhok city / Iraq.

The current study was carried out on a total of 157 patients, 89 females (57%) and 68 males (43%) who were clinically suspected of having pityriasis versicolor, attending the dermatological department of Azadi Teaching Hospital in Duhok city during the period from July 2024-October 2024. Dimethyl sulfoxide (DMSO) was used to dissolve antifungal drugs, which were then utilized to test antifungal sensitivity using the agar well diffusion method against widely used antifungal medications, including (itraconazole, fluconazole, ketoconazole, and nystatin). In this study the majority of patients were in the age group of 21-30 years. Within clinical signs, hypopigmented type of PV was the most predominant type (42.7%).

The majority of patients showed two or more areas of involvement which were seen in 57 cases (36.3%). More people in urban area (92.4%) were impacted than in rural areas (7.6%).

Regarding antifungal susceptibility, the most effective drugs were ketoconazole and nystatin, followed by itraconazole and finally fluconazole.

Keywords: Pityriasis, yeast, antifungal sensitivity, Duhok.

INTRODUCTION

A common superficial fungal infection of the skin is pityriasis versicolor (PV), also referred to as *Tinea versicolor.* On the trunk and upper arms, patients with tinea versicolor usually exhibit finely scaled, oval or round, hypopigmented or hyperpigmented macules or patches. Sometimes, especially when the condition is more widespread, patients report pruritus. Variable-colored skin lesions that may arise in this disorder are referred to as "versicolor." *Tinea versicolor* has a wide range of clinical manifestations and differential diagnoses (Goldstein *et al.,* 2018). The genus *Malassezia* (previously known as *Pityrosporum*) comprises dimorphic lipophilic and lipid-dependent yeasts, particularly *Malassezia globosa (M. globose), M. furfur,* and *M. sympodialis,* which are the cause of pityriasis versicolor. *M. japonica, M. obtuse, M. slooffiae, M. restricta,* and *M. pachydermatis* are among the other species that have been implicated (Nguyen *et al.,* 2020; Romero-Sandoval *et al.,* 2017).

These yeasts are typical commensals found on the epidermis. The prevalence of skin colonization rises with age; nearly all adults and 25% of children are impacted. When the saprophytic yeast or budding stage of the organism changes into the pathogenic hyphal or mycelial form pityriasis versicolor happens. The stratum corneum is where the fungal infection is located. A humid and warm environment, hyperhidrosis, applying greasy lotion or cream to the skin, excessive lipid-containing sebaceous secretions, malnourishment, poor general health, using oral contraceptives, pregnancy, diabetes mellitus, immunodeficiency, and genetic predisposition are all contributing factors for the conversion (Alam *et al.,* 2021; Kilinc *et al.,* 2018). Pityriasis versicolor is a global condition.

In regions that are hot and humid, the frequency is extremely high. The incidence can reach 50% in certain tropical nations. Teens and young adults are the most likely to experience it due apparently to their higher sebum production. Despite being rare, the illness can affect both young children and the elderly. *Tinea versicolor* in newborns and babies has been documented infrequently.

About 17% of afflicted people have a positive family history of tinea versicolor (Abdollahimajd *et al.,* 2019). Diagnosis is made on the basis of direct microscopic examination of scales, which show characteristic spherical spores and short curved hyphae "spaghetti and meatballs" appearance, as well as the clinical appearance of lesions (hypopigmented or hyperpigmented scaly macules).

According to the various species of *Malassezia,* there are reported differences in susceptibility to antifungal medications. Lack of quick and easy identification techniques could seriously affect the timely and appropriate administration of treatment, particularly in cases where nosocomial bloodstream infections are caused by *Malassezia* yeasts.

To verify the species identity by cultural approaches, various schemes have been developed based on the variation in fatty acid requirements among variants. The Japanese identifying system has been suggested recently. A variety of media, including Tween 60-Esculin agar, modified Dixon's agar, Sabouraud Dextrose Agar, and CHROMagar-*Malassezia* (CHROMM), must be used to subculture the isolated strain (Kaneko *et al.,* 2007). The chronicity and recurrence of *Malassezia* infections make treatment extremely difficult. Since the genus identity cannot predict susceptibility, the topical and systemic antifungal medications utilized to treat infection were unsatisfactory.

Antifungal therapy, however, is not clinically effective for infections caused by *Malassezia*, antifungal resistance in *Malassezia* species could be the cause of this (Romald *et al.,* 2020). The purpose of this study is to investigate the prevalence and *in vitro* antifungal susceptibility profile of *Malassezia* species that have been identified from PV patients.

MATERIALS AND METHODS

Samples collection

Following approval by the institutional ethics committee, the current study was carried out on a total of 157 patients who were clinically suspected of having pityriasis versicolor attending the dermatological department of Azadi Teaching Hospital in Duhok city during the period from July 2024-October 2024. The clinical diagnosis of PV was done by dermatologist using a wood’s lamp illuminated the skin lesion in a dark room to emphasize pigment changes in suspicious patients.

Following diagnosis and confirmation by the dermatology department physician, samples were collected from the patients. Each patient was given a unique questionnaire form that included information about their name, age, sex, occupation, family history, presence of an animal in their environment, the presence of any other chronic diseases like diabetes, cancer, etc., as well as clinical details like the location of the lesion, seasonal distribution, skin type, and whether they were hypopigmented or hyperpigmented Fig. (1). Moreover, a few of the patients who were examined reported various symptoms, particularly in the summer season, like itching and a tingling sensation.

Fig. 1: Pityriasis versicolor presenting as multiple hypopigmented, hyperpigmented, and erythematous macules and patches on different parts of patients' body.

Transport of samples

Under aseptic precautions, scales with repeated scraping were taken from the sites showing good fluorescence. They were obtained from chest, back, abdomen, arms, shoulders and neck area.

A sterile surgical blade was used to scrape the skin after using cotton soaked with 70% alcohol. The scales were collected in squares of clean, dark-colored paper. In cases where there is oily lesion a sterile cotton swab was used to collect specimen. In some PV patients who had more than two lesion sites, all lesions were sampled. Then collected swabs were preserved in an appropriate medium and all specimens including (Skin scrapings and swabs) transported at room temperature to the Mycology Laboratory Department of Microbiology at college of health sciences/ university of Duhok.

Direct microscopic examination (DME)

Direct microscopic examination of specimens was performed by placing them on a clean glass slide mounted with a drop of 10% potassium hydroxide (KOH) or lactophenol cotton blue (LPCB), covered with a coverslip with gentle pressure to prevent the formation of air bubbles. The slide was warmed gently and examined under low power (10X) and high power (40X) of light microscope for the presence of thick-walled rounded yeasts and short, stubby hyphae, which are characteristics of (spaghetti and meatballs), validated the pityriasis versicolor (PV) diagnosis (Leung *et al.,* 2022) Fig. (2).

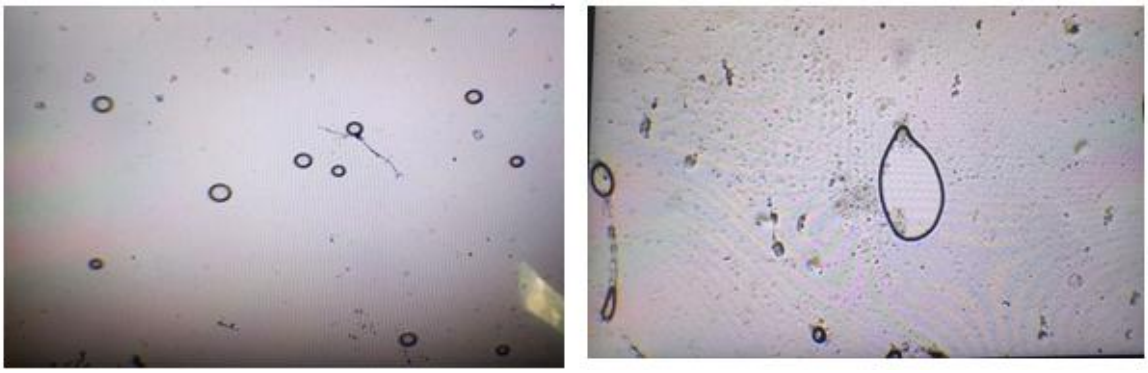

Fig. 2: Direct microscopic examination with 10% potassium hydroxide (KOH) and lactophenol cotton blue (LPCB).

Media used for primary isolation

After direct microscopic examination done for specimens, those samples that showed positive result with potassium hydroxide (KOH) or lactophenol cotton blue (LPCB), were subjected to culture. Using a sterile swab the surface of two SDA (Sabourad's Dextrose Agar) overlaid with sterile olive oil and plain SDA, two PDA (Potato Dextrose Agar) overlaid with sterile olive oil and plain PDA media containing amoxicillin (to inhibit bacterial growth) were streaked with the sample. Then all cultured plates were incubated at 37˚C for a maximum of 7-10 days and examined every day for growth. After that, all negative cultures were excluded, while positive cultures were examined both macroscopically and microscopically for further identification Fig. (3).

Fig. 3: Growth of *malassezia* species on PDA (A) and SDA (B).

Yeast identification

Yeast colonies that grew on primary isolations were subcultured on chromogenic agar (CHROMagar-*Malassezia*) LAB-M (AL019 HarlequinTM *Malassezia* Chromogenic Agar) using a sterilized loop and incubated at 30-37°C for 72 hours. It is a selective and differential culture medium that isolates and presumes the presence of clinically significant *Malassezia* species by using considerably diverse colony colors and morphologies. By the way, eight *Malassezia* species were identified in this study. Then microscopic examination for all species was performed by using gram stains after confirmation on CHROMagar-*Malassezia* Fig. (4).

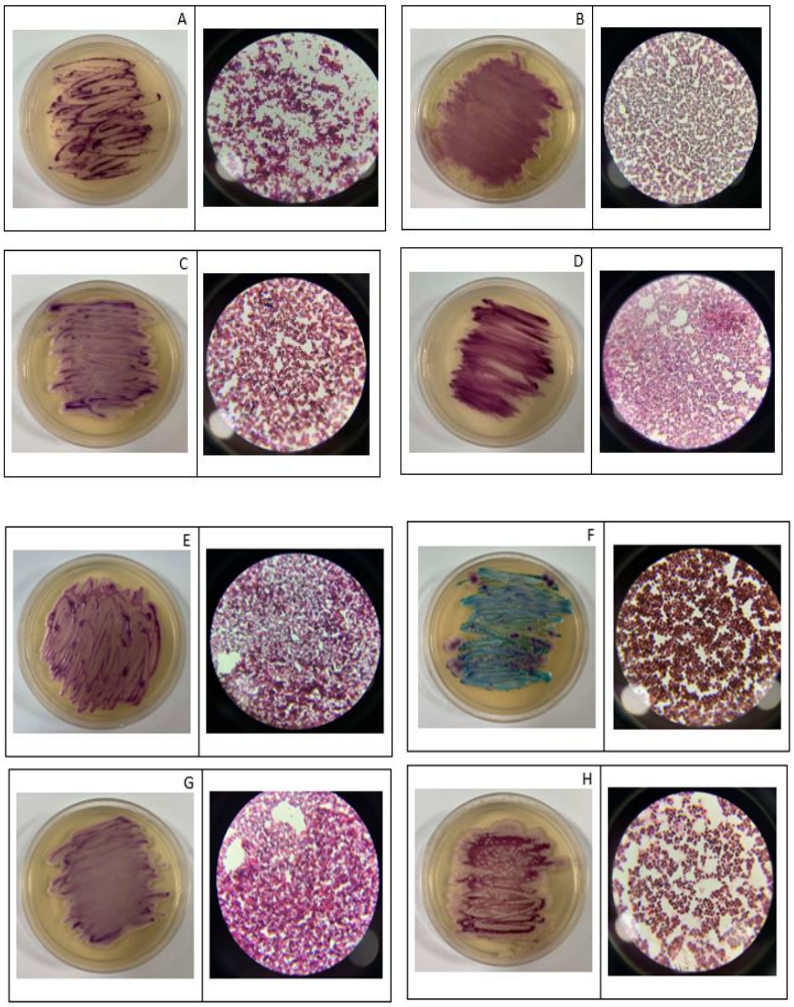

Fig. 4: Gram stain and growth of *malassezia* species on CHROMagar (A) *Malassezia furfur* (B) *M. pachydermatis* (C) *M. sympodialis* (D) *M. globosa* (E) *M. obtuse* (F) *M. restricta* (G) *M. slooffiae* (H) *M. dermatis*.

Urease test

This test was performed for the differentiation of *Malassezia* species on the basis of urease production using urea agar base. *Malassezia* isolates were inoculated on the surface of the urea agar slant, and incubated at 37˚C for 5-7 days. After incubation, a pink color was considered as a positive result, and yellow color as negative, all eight *Malassezia* species were urease positive Fig. (5).

Fig. 5: Urease test of *Malassezia* species using urea agar base.

Catalase test

A drop of 30% hydrogen peroxide solution was applied to a culture smear on a glass slide to detect the presence of catalase. The formation of gas bubbles, which indicate the release of oxygen, was regarded as a positive response. *M. restricta* is the only one of the eight *Malassezia* species that does not exhibit catalase activity Gueho et al (Guého *et al.,* 1996) Fig. (6).

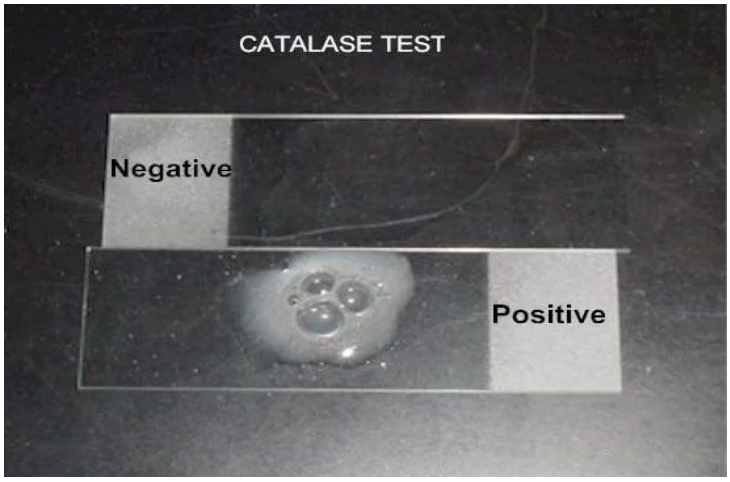

Fig. 6: Catalase test of *Malassezia* species.

Tween assimilation test

The ability of *Malassezia* to use tween compounds (Tween 20, Tween 40, Tween 60, and Tween 80) distinguishes them from one another. After melting 16 ml of sterile SDA and letting it cool to 50°C, 2 ml of a *Malassezia* yeast suspension containing roughly 105 CFU/ml was combined with the SDA, and the mixtures were plated using the pour plate method. Using a 2 mm diameter punch, four wells were created in the agar, one in each quadrant, and filled with five microliters of Tween 20, Tween 40, Tween 60, and Tween 80. The plates were incubated at 32°C for one week. The amount of tween used was determined by the lipophilic yeasts' growth and/or reaction (precipitation) surrounding the wells (Guillot *et al.,* 1996) (Table 1).

Table (1): Tween assimilation test for *Malassezia* species.

***Malassezia* species**	**Tween Assimilation**			
	80	**60**	**40**	**20**
Malassezia furfur	+	+	+	+
M. pachydermatis	+	+	+	+/−
M. sympodialis	+	+	+	−/+
M. globosa	−	−	−	−
M. obtuse	−	−	−	−
M. restricta	−	−	−	−
M. slooffiae	−	+	+	+
M. dermatis	+	+	+	+

Antifungal susceptibility test

Antifungal susceptibility test was performed for all specimens exhibiting positive growth for *Malassezia* species on both PDA and SDA. Eight *Malassezia* species isolates were examined for the antifungal susceptibility test after confirming its species on CHROMagar-*Malassezia*, each species then subcultured on SDA with chloramphenicol to obtain a pure colony, later inoculum suspensions

were made for each one. Antifungal drugs were used are (itraconazole 100 mg, fluconazole 150 mg, ketoconazole 200 mg, nystatin 500,000 IU). Antifungals stock solution was made by dissolving 50 mg of each antifungal medication in 2 ml of dimethyl sulfoxide (DMSO) to create concentration of 10,000 µg/ml stock solution, then 1ml of each was taken to create a serial dilution 1000, 100, and 10 µg/ml. The agar well diffusion method was used to conduct the antifungal susceptibility test, using a pipette 0.1 ml of fungal inoculum was first added to the surface of the agar MHA (Mueller Hinton Agar). The entire inoculum was then equally distributed across the entire plate using a sterile swab. Sterile corck borers were then used to create three holes in each plate measuring 6 to 8 mm in diameter. Each hole was then filled with 100 µl of the antifungal agents. Then, over 24 to 48 hours, the agar plates were incubated at 37°C. Finally, by measuring the inhibition zone, the susceptibility of each fungus to each antifungal drug was ascertained.

Preservation of the isolated strains

The preservation of all *Malassezia* spp. isolates were done by culturing in sabouraud dextrose broth (SDB) in sterilized screw glass tubes with or without olive oil enveloped with parafilm and stored at 5℃ in a refrigerator for further mycological testing.

Statistical analysis

The STATA intercooled version 12.1 was used to analyze the data (StataCorp. LP, College Station, TX). The mean, standard deviation, median, and range were used to express quantitative data. Numbers and percentages were used to present qualitative data.

RESULTS AND DISCUSSION

Out of 157 pityriasis versicolor (PV) patients, 89 cases were females (n=89; 57%) and 68 cases were males (n=68; 43%) Fig. (7). Involved individuals were in the age groups between 5 to 71 years old. Majority of them were in the age group of 21-30 years (34%), followed by 11-20 years (29%), 31-40 years (13%), 41-50 years (10%), 1-10 years (6%), 51-60 years (5%) and >60 years (3%) Fig. (8). The majority of the patients (42.7%) presented with hypopigmented type of PV while (31.9%) had hyperpigmented type of PV and (20.4%) patients had erythematous type and (5.1%) had mixed type Fig. (9). Most patients with hypopigmented PV were skin Type III and IV medium and moderate brown colors, while those with hyperpigmented lesion were pale skin Type I and medium type III.

Distribution of PV lesions on different sites of the body revealed that multiple lesions were seen in 57 cases (36.3%), these patients showed two or more areas of involvement, followed by back (19.7%), neck (14%), abdomen (8.9%), chest (7%), arms and shoulders (6.4%), face (5.7%), and underarms (1.9%) Fig. (10). In this study, 145 patients (92.4%) belonged to urban population while 12 patients (7.6%) were from rural areas, *Malassezia* infections were more common among patients who lived in urban areas. Family history of PV infection was present in 23 patients (14.6%) and remaining 134 patients (85.4%) had no such history. Regarding occupation of these patients, students were most frequently exposed to *Malassezia* infection 66 cases (42%), followed by housewives (24%), prisoners (13%), workers (8%), unemployed (5%), clerk (4%), peshmerga (2%), salesman (1%), and shepherd (1%).

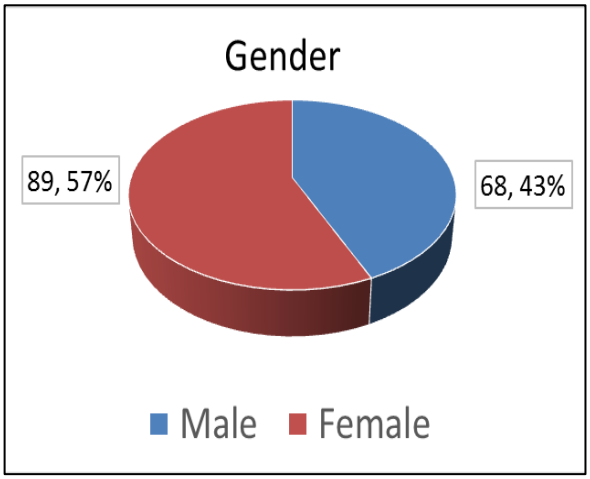

Fig. 7: Gender distribution in the study population (n=157).

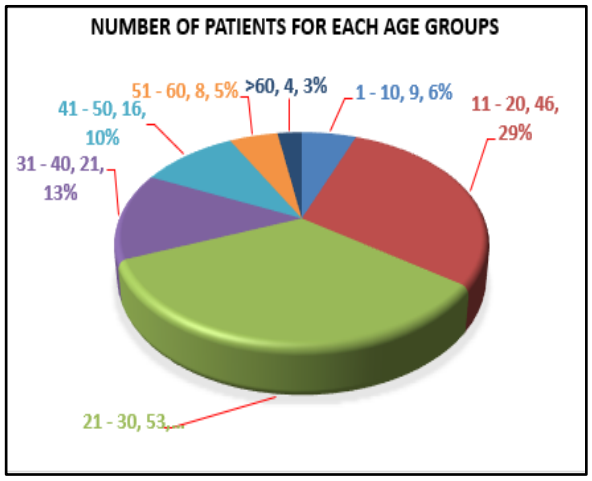

Fig. 8: Age distribution in the study population (n=157).

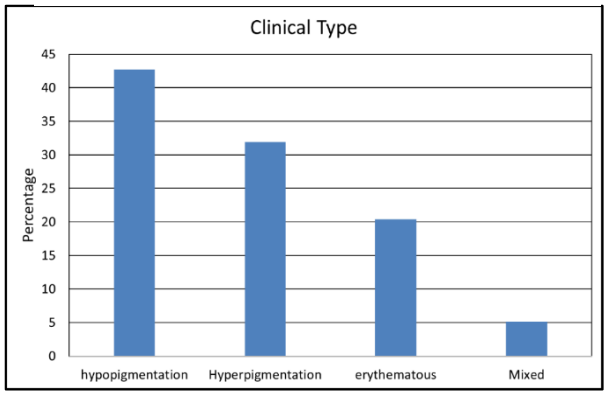

Fig. 9: Distribution of clinical types of PV among study group.

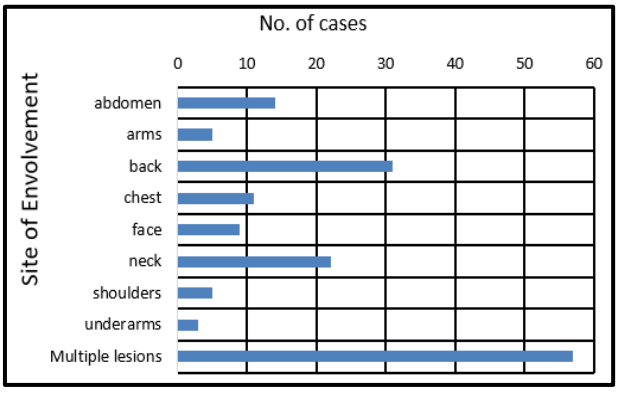

Fig. 10: Distribution of site of involvement of pityriasis versicolor patients.

A well diffusion approach was used to conduct an antifungal sensitivity test for the most widely used antifungal drugs, including (itraconazole 100 mg, fluconazole 150 mg, ketoconazole 200 mg, nystatin 500,000 IU). By measuring the inhibition zone, the susceptibility of each fungus to each antifungal drug was ascertained as shown in (Tables 2, 3, 4 and 5) and Fig. (11).

Table (2): Antifungal susceptibility test for Fluconazole.

***Malassezia* species**	**Fluconazole**		
	1000 µg/ml	**100 µg/ml**	**10 µg/ml**
Malassezia furfur	+++	+++	+++
M. pachydermatis	-	++++	++++
M. sympodialis	-	-	+++
M. globosa	++	-	-
M. obtuse	+	+	+
M. restricta	-	-	-
M. slooffiae	+	+	+
M. dermatis	+	-	-

+ mild sensitive
++ moderate sensitive
+++ severe sensitive
++++ very severe sensitive
- resistant

Table (3): Antifungal susceptibility test for Ketoconazole.

***Malassezia* species**	**Ketoconazole**		
	1000 µg/ml	**100 µg/ml**	**10 µg/ml**
Malassezia furfur	+++	+++	+++
M. pachydermatis	–	++++	++++
M. sympodialis	+	–	–
M. globosa	+++	+++	+++
M. obtuse	++	++	+
M. restricta	++++	+	+
M. slooffiae	++++	++++	++++
M. dermatis	+++	+++	+++

Table (4): Antifungal susceptibility test for Itraconazole.

***Malassezia* species**	**Itraconazole**		
	1000 µg/ml	**100 µg/ml**	**10 µg/ml**
Malassezia furfur	+++	+++	+++
M. pachydermatis	–	+	+
M. sympodialis	+++	+++	+++
M. globosa	++	–	–
M. obtuse	–	++	+++
M. restricta	++++	+++	+++
M. slooffiae	+++	+++	+++
M. dermatis	++	++	+++

Table (5): Antifungal susceptibility test for Nystatin.

***Malassezia* species**	**Nystatin**		
	1000 µg/ml	**100 µg/ml**	**10 µg/ml**
Malassezia furfur	–	++++	++++
M. pachydermatis	–	–	+
M. sympodialis	++++	+++	+++
M. globosa	+	+	–
M. obtuse	++++	+++	+++
M. restricta	++++	++++	++
M. slooffiae	++++	++++	++++
M. dermatis	++++	+++	+++

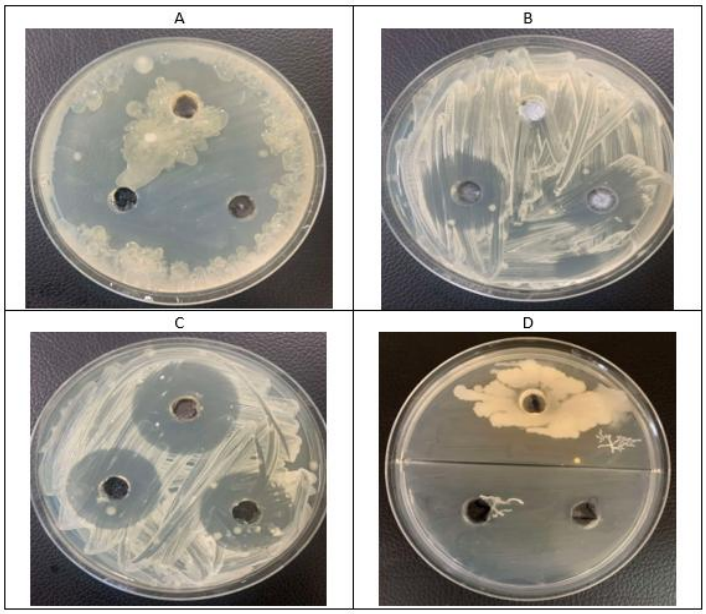

Fig. 11: Antifungal susceptibility test of *Malassezia* species A. Nystatin against *Malassezia furfur* B. Itraconazole against *Malassezia obtusa* C. Nystatin against *Malassezia obtusa* D. Ketoconazole against *Malassezia pachydermatis.*

Humans and other warm-blooded animals have resident skin flora that includes *Malassezia* species, which are found in (75-80%) of healthy subjects (Jang *et al.,* 2009). These yeasts are linked to a broad range of clinical symptoms, including benign skin disorders like pityriasis versicolor to fungemia in immunocompromised host (Shah *et al.,* 2013). In the current study, which was conducted at the Azadi Teaching Hospital in Duhok, Iraq, 157 patients suspected of having pityriasis versicolor PV were investigated, women were more likely than men to have PV (57%) compared to (43%). It is still unknown how sex affects a person's likelihood of developing PV. According to certain research, men are more likely than women to get PV (Erchiga *et al.,* 1999; Erchiga and Florencio, 2002).

However, some reports suggested that women are more likely than men to get these infections, which could be because women place a greater value on appearance and see dermatologists more frequently (Gupta *et al.,* 2001). The majority of infections were in women, which is attributed to women being exposed to pressures in their lives that are completely different from those in men, such as pregnancy and childbirth (Qassim *et al.,* 2024). As compared to other published data, the age groups of 21-30 and 11-20 years old had the highest prevalence of PV in our study this is probably due to increased sebum production throughout these years (Crespo-Erchiga *et al.,* 2008; Tarazooie *et al.,* 2004). Our findings are consistent with earlier research that found PV to be less prevalent in children, and we had fewer incidences of PV in the over-60-year age group according to different studies PV is infrequently observed in older people (Tarazooie *et al.,* 2004; Giusiano *et al.,* 2010). Hypopigmented lesions were seen in (42.7%), hyperpigmented lesions in (31.9%), erythematous lesions in (20.4%), and mixed lesions in (5.1%) of patients. The prevalence of hypopigmented lesions in PV was also demonstrated by other studies from India (Chaudhary *et al.,* 2010).

The fact that PV causes hypopigmentation in people with dark skin and hyperpigmentation in people with fair complexion. Research on patient pigmentary alterations revealed no relationship between pigment variation with the patient's age, sex, skin type, or lesion location. In our study, PV lesions are more commonly seen on multiple sites of the patient body that were seen in 57 cases (36.3%), these patients showed two or more areas of involvement, the higher density of sebaceous glands in certain areas may account for the higher incidence of involvement. The amount and distribution of sebaceous glands at a given location determine how lesions are distributed, followed

by back (19.7%), neck (14%), and less commonly other sites. Due to the fact that our center primarily serves urban and semi-urban populations, the majority of patients (92.4%) were from the urban population, this outcome was consistent with research by Snekavalli *et al.* (2018) that found that 65% of cases involved urban populations. A positive family history was found in (14.6%) of patients in accordance with the research conducted by Snekavalli *et al*. (31%) (Snekavalli, 2016**).** As we mentioned, the majority of the patients were students (42%) followed by housewifes (24%), where majority were students (29.09%), followed by housewives (20%) (Ghosh *et al.,* 2008). Regarding antifungal susceptibility testing that was performed using a well diffusion method on all eight *Malassezia* species it was demonstrated that all the isolates respond differently to all the tested antifungals with different drugs concentration, as shown previously in (Tables 2, 3, 4 and 5). The most effective drugs were ketoconazole and nystatin followed by itraconazole and finally fluconazole.

Some species were resistant to some antifungals and others were sensitive, and some were resistant to high concentration but sensitive to low concentration of drug and vice versa.

CONCLUSIONS

This is the first study in the Kurdistan region of Iraq focusing on the epidemiology of pityriasis versicolor (PV) in Duhok city. The results of our investigation showed that pityriasis versicolor was very prevalent in Duhok city. The age group most frequently impacted by PV was 21-30 years old, fewer of them in over-60-year age group. Females outnumbered the males. The most common clinical symptom among PV patients was hypopigmentation lesions. Majority of patients showed two or more areas of involvement. More people in urban area were impacted than in rural areas. Patients who are using the local saunas had poor personal hygiene practices with greasy skin and those who do hard works, which is typically linked to excessive perspiration, were more susceptible to contracting pityriasis versicolor. Therefore, maintaining proper personal hygiene may assist prevent recurrences.

Due to its high recurrence rate, pityriasis versicolor may need to be treated on an occasional basis with topical or oral treatments. Antifungal susceptibility testing is crucial since it will assist the dermatologist in selecting the proper antifungal drugs for the patient, which will increase patient compliance and lessen the chronicity of the condition.

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انتشار أنواع مالاسيزيا ومدى حساسيتها للأدوية المضادة للفطريات المعزولة من مرضى بيتيرياسيس فيرسيكولور في مدينة دهوك، العراق

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الملخص

يُعدُّ داء المِسْكِرَة (Pityriasis versicolor) فطرًا جلديًا سطحيًا مزمنًا، يُسبِّبُه خميرة *مالاسيزيا Malassezia*، ويتميز بظهور بقع متقشرة ومُتغيرة اللون، غالبًا على الطرف العلوي والمناطق الدهنية الأخرى. تهدف هذه الدراسة إلى تحديد انتشار أنواع *مالاسيزيا* وملف حساسيتها للأدوية المضادة للفطريات لدى مرضى داء المِسْكِرَة في مدينة دهوك، العراق.

تم إجراء الدراسة على 157 مريضًا، منهم 89 أنثى (57%) و68 ذكرًا (43%)، تم الاشتباه في إصابتهم بداء المِسْكِرَة، وراجعوا قسم الأمراض الجلدية في مستشفى آزادي التعليمي في مدينة دهوك خلال الفترة من يوليو 2024 إلى أكتوبر 2024 .تم جمع العينات باستخدام مسحات قطنية معقمة وشفرات جراحية، ثم نُقلت إلى مختبر الفطريات في كلية العلوم الصحية بجامعة دهوك لإجراء التحليل المجهري والماكروسكوبي المباشر باستخدام الزرع على أوساط أجار البطاطا دكستروز (PDA)، وأجار سابورود دكستروز (SDA)، و CHROMagar-Malassezi .تم استخدام ثنائي ميثيل سلفوكسيد (DMSO) لتذويب الأدوية المضادة للفطريات، التي تم اختبار حساسيتها باستخدام طريقة الانتشار في الآجار ضد الأدوية المضادة للفطريات الشائعة مثل: الإيتراكونازول، الفلوكونازول، الكيتوكونازول، والنستاتين. أظهرت الدراسة أن غالبية المرضى كانوا في الفئة العمرية بين21–30 سنة، بينما كان عدد المرضى في الفئة العمرية فوق 60 سنة أقل. أما بالنسبة للعلامات السريرية، فكان النوع المِسْكِري المبيض (hypopigmented) هو الأكثر انتشارًا بنسبة (42.7%) .أظهر معظم المرضى وجود منطقتين أو أكثر من الإصابة، حيث تم ملاحظة ذلك في 57 حالة (36.3%) كما تبين أن معظم المصابين كانوا من المناطق الحضرية (92.4%) مقارنة بالمناطق الريفية (7.6%). فيما يتعلق باختبار حساسية الأدوية المضادة للفطريات، كانت الأدوية الأكثر فعالية هي الكيتوكونازول والنستاتين، تليها الإيتراكونازول، وأخيرًا الفلوكونازول.

الكلمات الدالة: بتيرياسيس، خميرة، حساسية الأدوية، مضادات للفطريات، دهوك.