European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 02, 2020

Survey of *Malassezia* spp. that causing Pityriasis Versicolor in Al-Diwaniyah city, Iraq

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Abstract

Background: *Malassezia* spp. is part of the normal flora of the skin of human vertebrates and other warm-blooded vertebrates, it is associated with several diseases affecting human skin such as Pityriasis Versicolor (PV) which that often affects young adults of both females and males and can also affect children. The use of molecular methods has made great progress in the study of infections caused by *Malassezia* spp. because these species are variable in shape and difficult to identify according to colony shapes and micro and biochemical characteristics.

Materials and Methods: This study included 87 specimens, 38 of females, a percentage of 43.7%, and 49 specimens of males, a percentage of 56.3%, and the ages ranged between (10-55) years from patients that diagnosis by the dermatological consultant at Al-Diwaniyah Teaching Hospital for the period from 1/10/2019 to 5/3/2020.

Results: The results showed that the incidence of (PV) patients is the most common among patients aged 10-20 years, at a percentage of 45.9%, with significant differences at a probability level of 0.01, followed by patients between the ages of 30-21, at a percentage of 29.8% compared to other age groups. It was also observed, the incidence of (PV) is more common among patients with oily skin at a percentage of 60.9%, and the most isolated types are *M. furfur* and *M. globosa* for being lipophilic, followed by patients with normal skin at a percentage of 34.5% and patients with dry skin at a percentage of 4.6%. The results also showed that the incidence of (PV) is more frequent in patients who have a negative family history of the disease by 82.8% and those who do not have comorbidities, and by 94.3%, as for the status, duration, and severity of the disease, the results showed that the recurrent disease for less than 1 year of the severe type, it was the predominant among the other cases at rates of 58.6%, 41.4% and 37.9%, respectively. (PV) hypopigmentation also recorded the highest percentage among the infections with 54.0%, followed by hyperpigmentation at 39.1%, with significant differences compared to other injuries. The neck region is the most affected part of the rest of the body at a percentage of 36.8%, followed by the chest area by 23.5%, then the back area by 20.7%, and scaly infection was the highest percentage according to the type of infection, reaching 40.2%, followed by mixed infection, at 28.7%, with significant differences compared to other infections.

The results of the phenotypic and genotypic diagnosis of the growing colonies indicated that *M. furfur* was the most common causative agent (34.4%), followed by *M. globosa* (25.2%), *M. slooffiae* (18.3%), and *M. pachydermatis* at a percentage of (5.7%). The phylogenetic tree was also analyzed, and the similarity ratios between the local strains and the globally registered strains in the NCBI were compared using the MEGA10 program. The isolated local species were also registered in the Genbank and provided with the accession numbers.

Conclusions:

Malassezia furfur was the predominant causative agent of (PV) followed by *M. globosa*, *M. slooffiae*, and *M. pachydermatis*. Males and females are affected by the infection of (PV). Individuals of the age group between (10-20) years have oily skin, have a negative family history, the duration of the disease is less than 1 year, and the recurrent condition is most

affected by (PV). Scaly lesions with hypopigmentation affecting the neck were predominant in both genders.
Keywords: Survey, *Malassezia* spp., Pityriasis Versicolor.

Introduction

The fungus *Malassezia* spp. is one of the lipophilic yeasts known for more than a century as symbionts with the skin may be pathogenic under certain conditions (Kindo *et al*., 2004). It is part of the normal flora of the skin of human vertebrates and other warm-blooded vertebrates (Moniri *et al*., 2009). Being lipid-dependent, it is usually found in areas rich in sebaceous glands, and current evidence indicates higher rates of infection in healthy adults, in contrast to the low incidence in children before puberty (Juncosa *et al*., 2002).

Malassezia spp. is associated with several diseases affecting human skin such as Pityriasis Versicolor, folliculitis, seborrheic dermatitis, dandruff, steroid acne, atopic dermatitis, and psoriasis (Baroni *et al*., 2004; Wei *et al*., 2010). Although the fact of (PV) was first mentioned at the beginning of the nineteenth century, a great deal of controversy prevailed during the research and study of the etiological factors, due to the phenotypic differences and their difficult requirements for growth in the laboratory, and in 1801 the scientist Willan was the first to notice a disease. (PV) With increasing and strengthening studies, Baillon suggested in 1889 that the fungus *Malassezia* spp. it is the causative agent of (PV) (Ashbee and Evans, 2002).

Pityriasis Versicolor is an infection of *Malassezia* spp. in the skin, as it is sometimes called Tinea versicolor, although the term Tinea refers to infection with dermatophytes (Al-Saimary, 1993). Where the word Versicolor refers to a multi-colored rash, when this fungus grows, the skin disease (Pityriasis Versicolor) is produced. The appearance of a rash in the form of slightly scaly colored patches on the upper trunk, neck and arms, this occurs all over the world, and it is more common in hot and humid climates than in cold and dry climates, and this often affects people who suffer from excessive sweating and weakness in the system (Wu *et al*., 2015). (PV) often affects young adults of both genders and can also affect children, adolescents, and the elderly (Gupta *et al*., 2004). At first, these spots or pigment usually appear in the rich area. In the sebaceous secretory gland (neck, chest, arm, scalp, trunk, face, and shoulders) the most common types of *Malassezia* spp. associated with (PV) are *M. globosa*, *M. sympodialis*, and *M. furfur* (kindo *et al*., 2004). The use of molecular methods has made great progress in the study of infections caused by *Malassezia* spp. because these species are variable in shape and difficult to identify according to colony shapes and micro and biochemical characteristics. Therefore, molecular diagnosis are used to reduce the time, effort, and cost of diagnosis and because of the increase in (PV) in recent times, the aim of this study include isolation and identification of *Malassezia* spp. based on phenotypic and molecular methods, and the distribution of infections according to age, genders, skin type, family history, comorbidities, status, duration, and severity of the disease, location, pigmentation, and type of infection.

Materials and methods

Specimens Collections

87 specimens (49 males and 38 females) were collected from patients suffering from (PV) that diagnosis by the dermatological consultant at Al-Diwaniyah Teaching Hospital for the period from 1/10/2019 to 5/3/2020. Forceps, a surgical blade, and sterile slides were used to collect the skin specimens, and then they were transferred in sterile containers to the fungal laboratory in the Department of biology/College of Science, and then the distribution of infections according to age, gender, skin type, family history, comorbidities, status, duration, and severity of the disease, location, pigmentation, and type of infection.

Direct examination

Scraping specimens were prepared for direct examination by placing them on a clean glass slide and mixing them with a solution of (10%) KOH (to dissolve the keratinous substance), covered with the slide cover, and then gently heated the slides to prevent (KOH) crystallization and examined under the (X40) microscope (McPherson and Pincus, 2017).

Indirect examination

Cultivation of Specimens

All collected clinical specimens were cultured in sterile Petri dishes and using different culture media prepared for this purpose include SDA, mDA, and mLNA containing 0.04 ml Penicillin, 2 ml Streptomycin, 0.05 g Chloramphenicol, and 0.5 g Cycloheximide is added to olive oil or without olive oil. Scraping specimens were cultured by scattering them on the culture media. Then all specimens were incubated at (30-32 °C) for two weeks, and after the results of the culture appeared, the colonies were examined morphologically and microscopically and then purification (Boekhout *et al*., 2010).

Molecular diagnostics using PCR assay

A PCR assay was performed for *Malassezia* spp. for 18S rRNA gene ITS region, the examination consists of several steps, as follows:

1- Fungus DNA extraction

DNA extraction was performed from fungus colonies using the EZ-10 Spin Column Fungal Genomic DNA Mini-Preps Kit provided by the Korean company Bioneer, and the extraction was performed according to the company's instructions.

2- Primers

The specific primers are designed for the 18S rRNA gene ITS region of *Malassezia* spp. in this study using the Genbank NCBI website and by the Primer3plus program. The primers were prepared by the Scientific Researcher Company in Iraq/Al-Diwaniyah:

Table 1. Primers used in this study with their nucleotide sequence and PCR test product

Primer name	Sequence	Amplicon	Source
F	5'-TATCCACAAACCCGTGTGCA-3'	404bp	Designed for this s
R	5'-CCTCCCTTTCAGAGCGGTTT-3'		

3- Prepare the PCR master mix

The PCR reaction mixture was prepared using the AccuPower® PCR PreMIX kit prepared by the Korean company Bioneer according to the manufacturer's instructions and as in the following table:

Table 2. PCR master mix

PCR master mix	Volume (µl)
DNA template	5
Forward primer (10pmole)	1.5

Reverse primer (10pmole)	**1.5**
Free nuclease water	**12**
Final volume	**20**

After that, the components of the PCR reaction mixture mentioned in the above table were placed into 0.2 ml tubes of the Accupower ® PCR PreMIX kit containing the rest of the components of the PCR reaction, and then all tubes were transferred to the Vortex centrifuge. Exispin at 3000 rpm for three minutes and then placed in the Thermocycler PCR machine for amplification.

4- Thermocycler PCR conditions

The PCR examination was performed using a PCR Thermocycler device for the isolates under study and according to the method of Spadaro *et al*., (2012) as in the following table:

Table 3. Conditions of thermal cycles for PCR examination

PCR Step	Repeat cycle	Temperature	Time
Initial denaturation	**1**	**95°C**	**5min**
Denaturation	**35**	**95°C**	**30sec**
Annealing		**5°C**	**30sec**
Extension		**72°C**	**30sec**
Final extension	**1**	**72°C**	**5min**
Hold	**-**	**4°C**	**Forever**

5- DNA-base sequence-sequence analysis

DNA base sequence analysis was performed to diagnose *Malassezia* spp. This is done by sequencing the nitrogenous bases of the 18s rRNA gene ITS region and drawing the Phylogenetic tree by sending the product of the PCR reaction to Macrogen in South Korea to perform the DNA sequencing using the AB DNA sequencing system program. After obtaining the sequence, it was analyzed and matched with its counterparts at the site NCBI-BLAST identity, record the result at the Submission NCBI-Genbank site, and perform a phylogenetic tree analysis using MEGA10 software by comparing the DNA sequences of some local *Malassezia* spp. isolates with global isolates registered in the National Center for Biotechnology Information database (NCBI).

Statistical Analysis

The results of the study were analyzed statistically using the statistical program known as the Statistical Package for Social Sciences (SPSS, version 25). The significant differences were identified at 5% and 1% probability levels (McDonald, 2014).

Results and Discussion

Clinical and microbiological study

1- Demographic characteristics of patients with Pityriasis Versicolor

During the study period, 87 specimens were collected, 73 specimens (84%) were positive while 14 specimens (16%) were negative to direct and indirect examination.

2- Distribution of (PV) according to gender and age groups

The results of the statistical analysis of the current study showed that there were no significant differences between the incidence of (PV) between males and females at P> 0.05, as the infection rate in males was 56.3% and in females, 43.7% (Table 4), and the reason can be attributed to that the pathogens of (PV) are present on the surface of the body naturally for both genders, it becomes pathogenic due to the influence of environmental conditions such as high air temperature, high humidity, genetic predisposition, pregnancy, oily skin, hyperhidrosis, lack of hygiene, poor nutrition and weak immunity caused by chronic infections or the use of immunosuppressive drugs and cortisone and the use of oily preparations and creams (Theelen *et al*., 2018). These results are consistent with findings (Belec *et al*., 1991; Rasi *et al*., 2009; Framil *et al*., 2011).

The distribution of patients with (PV) was greater in the age group (10-20) years in both genders by 45.9%, followed by the age group (30-21) with 29.8%, then the age group (40-31) with a percentage of 14.9%, and finally the age group (more From 40) a percentage of 9.1% with significant differences between them at a probability level of 0.01 (Table 4). These results are in agreement with Santana *et al*., (2013) who found that the age group (10-20) years, which is the youth and adolescence stage, is most vulnerable to infection, and the reason for this is due to the high level of physical activity, hormonal changes and increased lipid activity with the production of sebum from the glands. Fatty acids allowing a high-fat environment in which *Malassezia* spp. (Komba and Mugonda, 2010).

Table 4. demographic characteristics of patients with (PV)

Variables		Total		P Value
		No.	(%)	
Genders	Females	38	43.7%	0.095***
	Males	49	56.3%	
Age (year)	10-20	40	45.9%	0**
	21-30	26	29.8%	
	31-40	13	14.9%	
	Over 40	8	9.1%	

** There are statistically significant differences at a probability level of 0.01.

*** There was no significant difference at p> 0.05.

3- Distribution of (PV) according to clinical features of individual

The results of the study of clinical characteristics and disease signs according to the questionnaire designed for the current study and shown in Table (5, 6) showed that (PV) is more common among patients with oily skin by 60.9% and with significant differences from the rest of the skin types at a probability level of 0.01, and the results also showed that most patients with (PV) do not have a positive family history of the disease (negative history) with a percentage of 82.8% and significant differences compared to patients with a positive family history of 17.2%.

It was also noted that patients who did not suffer from comorbidities such as allergies and psoriasis, their percentage was the highest compared to patients who suffer from comorbidities, reaching 94.3% with significant differences from the rest of the percentages, which amounted to 3.4% for allergies and 2.3% for psoriasis. In oily skin, because most *Malassezia* spp. Are lipophilic and have a high enzyme activity to digest these fats and cause infection (Crespo-Erchiga, 2002; Cermeño *et al*., 2005). Likewise, this disease is non-communicable, and that the occurrence of infections sometimes in more than one person within the same family may be attributed to the presence of a genetic factor for the infection (Hafez and Shamy, 1985).

Table 5. Clinical characteristics of the study individuals

Variables		Total No.	Total (%)	P Value
Skin Type	**Dry**	**4**	**4.6%**	**0****
	Oily	**53**	**60.9%**	
	Normal	**30**	**34.5%**	
History Family	**Positive**	**15**	**17.2%**	**0****
	Negative	**72**	**82.8%**	
Associated diseases	**Allergy**	**3**	**3.4%**	**0****
	Psoriasis	**2**	**2.3%**	
	None	**82**	**94.3%**	

** There are statistically significant differences at a probability level of 0.01.

The results also showed in Table (6) that the recurrent disease status of patients with (PV) was higher and by 58.6% than the recent infection by 41.4% of patients with (PV) for the first time, with significant differences (P <0.05) at a probability level of 0.05. These results are consistent with the findings of Framil, (2011); Morais *et al*., (2010) who observed the frequency of patients (52.6% and 67.4%, respectively). This is unexpected, given that severe or recurrent skin diseases may harm the psychosocial state of individuals (Charan, *et al*., 2013; Ibler and Jemic, 2013). (PV) generally disappears with treatment, but it often recurs when conditions are appropriate for its reproduction, such as hyperhidrosis and the use of body oils, as recurrence of infection is inevitable, but it is not necessarily that it occurs annually, but the infection may return once the causes are renewed, helping the activity of the pathogen. Therefore, the treatment must be completed completely in addition to taking some measures to prevent its recurrence, and for those who happen to them frequently, anti-fungal shampoo or anti-fungal treatment can be prescribed orally for one to three days every month. Sometimes the white marks persist for a long time after the scale and yeast are gone, and despite exposure to sunlight. In such cases, additional anti-fungal treatment is not helpful as it tends to be chronic and recurrent (Gaitanis *et al*., 2012).

As for the duration of the disease, the results showed that a group of less than one year was the most susceptible to developing Pityriasis Versicolor, with significant differences at a probability level of 0.01 compared to the other groups, as it reached 41.4%. (Gupta *et al*., 2003). The results of the study agree with the findings of Talaee *et al*., (2014) that the duration of (PV) was less than 6 months 44%, 6 months - 1 year 26%, then 1-2 years 10%.

As for the severity of the disease, the results showed no significant differences P> 0.05 in coverage of pigmentation ratios, which were classified into three categories according to the severity of the disease: Category 1: slight as pigmentation covered 20% of the patients' body surface and included 29.9% of the total specimens and Category 2: The severity of the disease was moderate over 30% of the body of the patients and included 32.2%. As for Category 3: the disease was severe, and the pigment covered more than 50% of the patients' skin and included 37.9%.

Table 6. Distribution of (PV) according to signs of disease

Variables		Total No.	Total (%)	P Value
	Recent	**36**	**41.4%**	**0.023***
	Recurrent	**51**	**58.6%**	

Mode of the Disease				
Duration of the Disease	**Less than 1 Y**	**36**	**41.4%**	**0****
	1-3 Y	**30**	**34.5%**	
	3-5 Y	**16**	**18.4%**	
	Over 5 Y	**5**	**5.7%**	
Severity of the Disease	**Mild**	**26**	**29.9%**	**0.510*****
	Moderate	**28**	**32.2%**	
	Severe	**33**	**37.9%**	

*There are statistically significant differences at a probability level of 0.05.
** There are statistically significant differences at a probability level of 0.01.
*** There was no significant difference at p> 0.05.

4- Distribution of (PV) according to site, pigmentation, and type of lesion on the Body Surface

The results in Table (7) showed the prevalence of (PV) on the upper parts of the body (neck, face, chest, back, abdomen, arm, and trunk) and the presence of (PV) on the neck was 36.8% with significant differences at a probability level of 0.01 from the rest of the sites, which is the chest. 23%, back 20.7%, face 9.2%, trunk 4.5%, arm 3.4%, then abdomen 2.3%, the explanation of the prevalence of infection in the upper body due to the presence of a high percentage of fat, compared with the lower body parts, may be related to the lipophilic nature Of the pathogen, as these areas are characterized by dense and active sebaceous glands (Varada *et al*., 2014).

As for the pigmentation of the incidence, Table (5), (PV) patients with hypopigmentation (loss of black or dark color) had a higher rate compared with the rate of hyperpigmentation (increased white skin color), which was 54.0% to 39.1%, respectively, with significant differences from the types of Other pigmentation is at 0.01 likelihood level.

The infection spots are circular, oval, or irregular in shape and multiple sizes, and their color varies from person to person, it may appear light in color in people with dark skin and may appear brown in people with white skin and its color may range between yellow, brown and reddish-brown, but it often takes a color. Paler than the rest of the skin color, these spots are covered with a superficial layer of scales similar to bran (Rai and Wankhade, 2009).

One theory refers to a tryptophan-dependent metabolic pathway that yeasts stimulate enlarged melanosomes (pigment granules) within the basal melanocytes and are responsible for the browning of the Pityriasis Versicolor. The yeasts are easier to show in the skimmers from this type of (PV) than those taken from the type. The white, white, or hypopigmented type of (PV) is thought to be due to a chemical produced by *Malassezia* spp. They spread in the epidermis and impair the function of melanocytes. (Thoma *et al*., 2005)

The explanation for the occurrence of skin discoloration (pigmentation condition) by (PV) may also be due to the degradation of melanin, and this is in agreement with many studies that have indicated this, including hypopigmentation, damage to melanocytes, and inhibition of Tyrosine by carboxylic acid, especially azelaic acid, produced by *M. furfur*, and most of the modifications occurred based on the cytotoxicity of carboxylic acids, as for the hyperpigmentation it is not fully understood, but it may be due to the increased thickness of the keratin and the infiltration of inflammatory cells more clearly in these individuals as an aid to the melanocytes (Acharya and Jayawali, 2017). Our results are in agreement with their findings (Hassan *et al*., 2009; Ibekwe *et al*., 2015; ElShabrawy *et al*., 2017). They found that hypopigmentation (white) was higher than hyperpigmentation (dark brown). Also, the results of the study agree with Talaee *et al*., (2014) that the neck and chest area, respectively, are the

most affected parts because the areas covered with clothes encourage the development of infection, which supports the concept that blockage of glands plays a role in this disease.
The results, Table (7) also showed that there are significant differences at the 0.01 probability level between the types of infection, as the rate of scaly infection reached 40.2%, followed by the mixed infection at 28.7% and the feeling of itching by 24.1%, which resembles a pin spoke, especially when the temperature rises. *Malassezia* spp. has ability to produce several enzymes, including the protease enzyme (Continuho and Paula, 2000; Mancianti *et al*., 2001). Protease can contribute to itching as a mediator (Chen and Hill, 2005). Or the cause of itching may be due to the effect of dryness in the stratum corneum from increased sweating, and fine scales may not be easily visible on the infection, but it is easily obtained when the skin is easily tightened or Santana *et al*., 2013).

Table 7. Distribution of (PV) according to the characteristics of the infection

Variables		Total No.	Total (%)	P Value
Lesion Site	Neck	32	36.8%	0**
	Face	8	9.2%	
	Chest	20	23.5%	
	Back	18	20.7%	
	Abdomen	2	2.3%	
	Arms	3	3.4%	
	Trunk	4	4.5%	
Lesion Pigmentation	Hyper	34	39.1%	0**
	Hypo	47	54.0%	
	Erythematous	2	2.3%	
	Hyper & Hypo	4	4.6%	
Lesion Type	Itchy	21	24.1%	0**
	Scaly	35	40.2%	
	Mixed	25	28.7%	
	None	6	6.9%	

** There are statistically significant differences at a probability level of 0.01.

Isolation and identification of *Malassezia* spp.

Polymerase chain reaction (PCR)

PCR technique was adopted in the diagnosis of some fungal isolates under study (13 isolates) by using specific primers for the 18S rRNA gene ITS region, it was observed after electrophoresis on the gel of 1% agarose and examination under radiation the presence of a single band of DNA with a molecular size of 440 base pairs, and these results indicate the genus *Malassezia* spp. (Figure 1).

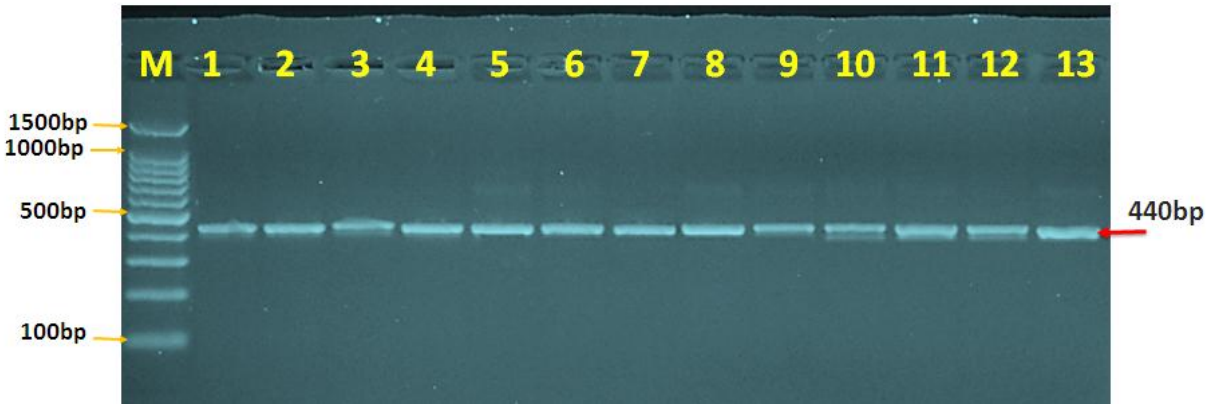

Figure (1) Electrophoresis using agarose gel 1%**, shows the results of the PCR examination of the 18S rRNA gene ITS region for the diagnosis of *Malassezia* spp. M: Marker ladder (100-1500bp), Lanes (1-13) positive fungus isolates with a product of 440 bp.**

DNA sequencing technique

After the completion of the PCR test, the product of PCR was sent to Macrogen in South Korea for DNA sequencing, using the AB DNA sequencing device, and then compared with the DNA sequence of some *Malassezia* spp. Registered on the NCBI site using the MEGA10 program, as well as the analysis of the phylogenetic tree of the type (Test UPGMA tree) was performed to clarify the evolutionary relationships between the species, which helps in diagnosing the species whose classification is suspected. The results of the analysis showed a clear convergence between the species under study with the other recorded species in the site, which appeared in the phylogenetic tree analysis (Fig. 2), had high matches for the sequence of nitrogenous bases of local and global isolates (Table 8). The results of the analysis of nitrogenous bases of local isolates were recorded in the Genbank site with accession numbers.

Table 8. The NCBI-BLAST Homology Sequence identity (%) between local *Malassezia* spp isolates and NCBI-BLAST submitted *Malassezia* spp:

***Malassezia* spp - No.**	**Genbank Accession number**	**NCBI-BLAST Homology Sequence identity (%)**		
		Identical Malassezia species	**Genbank Accession number**	**Identity (%)**
***Malassezia* spp. isolate No.1**	**MW016930**	***Malassezia slooffiae***	**JQ699099.1**	**99.43%**
***Malassezia* spp. isolate No.2**	**MW016931**	***Malassezia furfur***	**LC317631.1**	**99.75%**
***Malassezia* spp. isolate No.3**	**MW016932**	***Malassezia furfur***	**LC317631.1**	**99.75%**
***Malassezia* spp. isolate No.4**	**MW016933**	***Malassezia globosa***	**KM269142.1**	**100.00%**
***Malassezia* spp. isolate No.5**	**MW016934**	***Malassezia furfur***	**LC317631.1**	**99.75%**
***Malassezia* spp isolate No.6**	**MW016935**	***Malassezia furfur***	**LC317631.1**	**99.49%**
***Malassezia* spp. isolate No.7**	**MW016936**	***Malassezia globosa***	**KM269142.1**	**99.75%**

***Malassezia* spp. isolate No.8**	**MW016937**	***Malassezia furfur***	**LC317631.1**	**99.75%**
***Malassezia* spp. isolate No.9**	**MW016938**	***Malassezia pachydermatis***	**KX196171.1**	**99.56%**
***Malassezia* spp. isolate No.10**	**MW016939**	***Malassezia furfur***	**LC317631.1**	**100.00%**
***Malassezia* spp. isolate No.11**	**MW016940**	***Malassezia pachydermatis***	**KX196171.1**	**100.00%**
***Malassezia* spp. isolate No.12**	**MW016941**	***Malassezia furfur***	**LC317631.1**	**99.14%**
***Malassezia* sp isolate No.13**	**MW016942**	***Malassezia furfur***	**LC317631.1**	**99.50%**

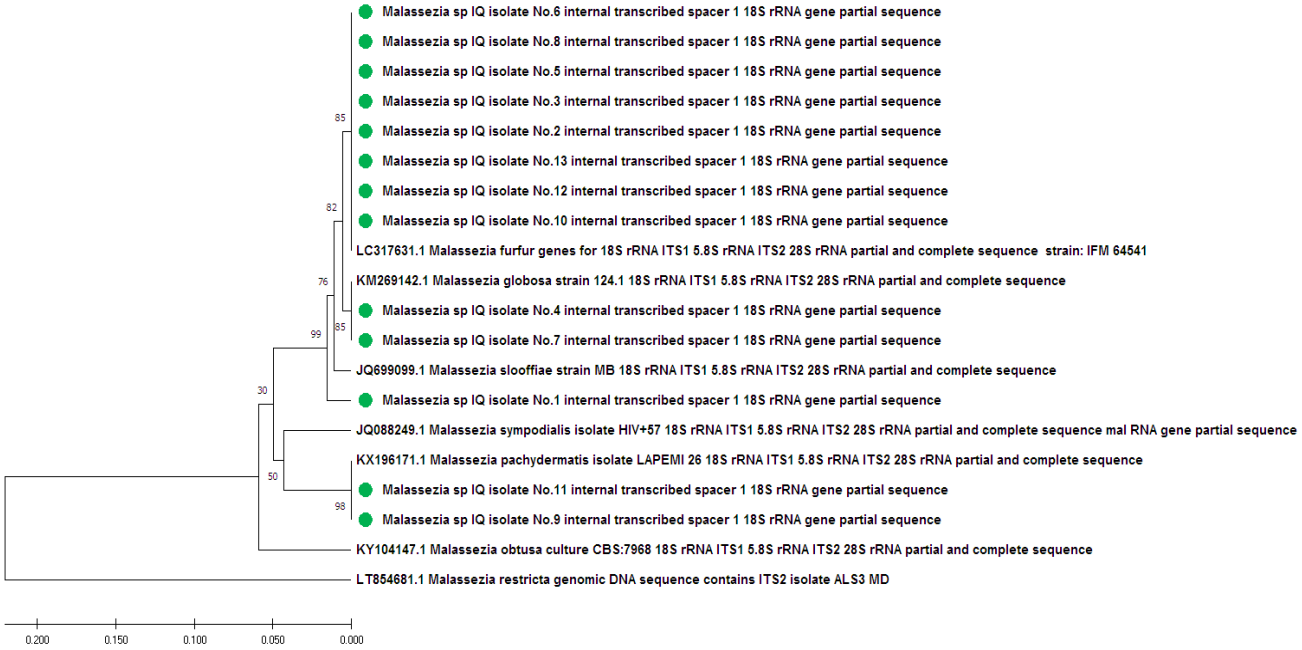

Figure (2) Test UPGMA tree for *Malassezia* spp. Using MEGA10 software

The design of primers to amplify different regions of rDNA has great merit in the taxonomic studies of fungi, especially the designed primers based on the ITS region by which many ITS region sequences are detected and for different fungi and have been used mainly to study the genetic relationships between different species within the genera or physiological forms dependent for one species (Mitchell *et al*., 1995).

Shokohi *et al*., (2009) distinguished between some species of *Malassezia* spp. by amplifying the ITS1-5.8S rRNA gene- ITS2 region, and these are *M. furfur, M. globosa, M. sympodialis, M. restricta,* as well. Awad *et al*., (2019) was able to diagnose some species of *Malassezia* spp. include *M. furfur M. globosa, M. pachydermatis* isolated from animals (dogs) and humans by amplifying the ITS region, the molecular size of the DNA was 509-430 base pairs.

The final results of isolation and identification of *Malassezia* spp. based on direct microscopic examination of specimens with 10% KOH, and indirect methods of cultivating specimens on appropriate culture media and molecular methods confirm that *M. furfur* it was most apparent from the other species, with significant differences at a probability level of 0.01, as 30 isolates were isolated with 34.4%, followed by *M. globosa* with 22 isolates at 25.2%, then *M. slooffiae* with 16 isolates with 18.3% and *M. pachydermatis* with 5 cases, 5.7%. While the number of

specimens that did not give positive growth was 14 specimens, at a percentage of 16.0% of the total number of specimens, 87 specimens (Table 9).

Table 9. *Malassezia* spp. Isolated from patients with (PV)

***Malassezia* spp.**	**Total**	
	No.	**(%)**
M. furfur	**30**	**34.4%**
M. globosa	**22**	**25.2%**
M. slooffiae	**16**	**18.3%**
M. pachydermatis	**5**	**5.7%**
Negative	**14**	**16.0%**
P-value	**0****	

** There are statistically significant differences at a probability level of 0.01.

Conclusions

Malassezia furfur was the predominant causative agent of (PV) followed by *M. globosa*, *M. slooffiae*, and *M. pachydermatis*. Males and females are affected by the infection of Pityriasis Versicolor. Individuals of the age group between (10-20) years have oily skin, have a negative family history, the duration of the disease is less than one year, and the recurrent condition is most affected by Pityriasis Versicolor. Scaly lesions with hypopigmentation affecting the neck were predominant in both genders, Fungal isolates of species. *Malassezia* spp. Were identical with the reference isolates when using the DNA sequencing technique. The evidence shown by the phylogenetic tree showed that all species have high similarity with each other.

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