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Isolation and identification different type of fungi from skin in Tikrit city

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ABSTRACT: This study was conducted in the postgraduate laboratory at the College of Science at Tikrit University from October 2023 to May 2024 with the aim of investigating fungi causing skin diseases Dermatomycosis. 100 samples were collected from patients visiting the dermatology consultant at Tikrit Teaching Hospital, Al-Elm General Hospital and some private clinics in Tikrit city for ages between 1 - 70 years. Laboratory culture showed positive results at a rate of 50% of the total samples, as the results showed that the most type of skin fungus isolated during the study was the fungus Trichophyton mentagrophytes at a rate of (16%) of the total number of positive samples, followed by the fungus Microsporum canis at a rate of (12%), while Candida albicans appeared at a rate of (10%), followed by Candida tropicals at a rate of (8%), Candida parapsilosis at a rate of (6%) and C. lipolatica at a rate of (4%), while T. verrucosum and Microsporum gypseum appeared at a rate of 2% each, and the molds Aspergillus niger and Aspergillus tubingenesis appeared at rates of (8%, 6%) each, respectively. The results showed that scalp ringworm (20%) and finger ringworm (16%) were the most common among patients, with the percentage of males (60%) higher than females (40%), and the ages of 10-30 years were the most affected at 40%.

Keywords: *BMP-15*, reproductive hormones, Real time PCR, Mini VIDAS

1. INTRODUCTION

The skin is one of the most important organs of the body and a mechanical defensive barrier that prevents pathogens and foreign substances from penetrating the body and causing infection(1). Dermatophytes are known as keratinophilic fungi due to their affinity for the keratinophilic tissue that makes up the skin, hair, nails, horns, hooves, feathers and wool as a natural habitat (2). They cause infections known as ringworm or tinea (3). This term is used for fungal infections that start as a small rash and then spread in a ring-like shape because the fungus grows evenly in all directions, forming a circular ulcer on the skin or scalp that resembles the holes made by moths in clothes (4). There are three genera of keratinophilic fungi: Trichophyton, Epidermophyton, Microsporum (5)

The main feature that made dermatophytes important from a medical point of view is their ability to decompose natural solid keratin and exploit it as a food substance in their motile phase to benefit from proteins as a food source, as these fungi tend to use amino acids, peptides and proteins as sources of carbon and nitrogen together for growth and

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reproduction, so these fungi are described as keratinolytic and keratinolytic by possessing many virulence factors that qualify them to penetrate keratinous tissues such as skin, hair and nails. The most important of these enzymes are protease, phosphatase, lipase, collagenase, esterase, phospholipase, gelatinase, cellulose and keratinase (6).

Keratinophilic fungi are mostly commensal unless certain, yet poorly defined, factors trigger the transformation into a pathogenic state. Some of the factors that trigger such a transformation can depend on the fungal species, the environment, the immune status of the individual, and most importantly, the host's genetics (7).The immune mechanisms for defense against fungal infections are numerous, ranging from protective mechanisms that were present early in evolution (innate immunity) to sophisticated adaptive mechanisms that are specifically triggered during infection and disease (8) The present study aimed to Isolation and identification of fungi causing skin infections.

2. MATERIALS AND METHODS

2.1 Subjects

100 samples were collected from both sexes, aged between (1-70) years, where samples were taken from people infected with skin fungi. They were collected in the Dermatology Consultant at Tikrit Teaching Hospital, Al-Alam General Hospital and outpatient clinics, where samples were taken from the infected areas (head, skin, nails). The collection of samples took from October 2023 to May 2024, and a questionnaire form was allocated that included information about the infected person (name, age, gender, residence, phone number, date of taking the sample, date of infection, accompanying symptoms, type of infection, place of obtaining the sample).

2.2 Sample collection

1. Collecting samples from skin: The samples were taken from the skin after sterilizing the infected areas using 70% ethanol alcohol to get rid of bacteria and remove the materials stuck in the skin and ointments, then scraping the infected area Active border because it contains fungal threads using a sterile surgical blade and placing the crusts in a sterile container for the purpose of culturing them on saproid dextrose agar medium and the plates were incubated at 35°C for two to three weeks (AL-Dorry, 1980).

2. Collecting samples from the scalp: Sterile forceps were used to collect hair and crust samples from the spreading edge of the infected area on the head and the method mentioned above in paragraph 1 was followed.

3. Collecting samples from nails: The samples were taken from the infected nails by cutting the upper edges of the infected nail, and the keratinized remains collected under the infected nail were also collected, and the method mentioned above in paragraph 1 was followed.

Isolation and identification

The samples were directly cultured in SDA medium and incubated at 35°C for 2-3 weeks and the plates were continuously examined every 2-3 days of incubation to observe the growth (9).After the appearance of fungal growth on the medium, the fungus and its type were diagnosed by performing the following:-

1. Morphology examination of the colonies

The morphological examination of the dermatophyte colonies included the shape of the colony, the color of the colony, its texture (cotton, powdery or downy), the incubation period and examining it from the opposite side (10). The morphological examination is one of the important tests to distinguish dermatophytes from others.

2. Microscopically examination of the colonies

This examination was performed by placing a drop of LPCB-cotton blue stain on a glass slide using a sterile inoculation needle. Then, a portion of the fungal hyphae was transferred from the fungal colony to the glass slide, mixed with the stain, and the slide cover was placed on it and gently pressed to brush the sample and left for 2-5 minutes, then dried over a low flame. After that, it was examined under the microscope using power (x10) and then power (x40) to observe the shapes, sizes, spores, and type of conidia (11)

2.3 Statistical analysis

The statistical analysis of the results was conducted using the Statistical Package Social Science (SPSS) test, and significant differences were determined using the Chi-square test, with significant levels at >0.05 (12).

3. RESULTS AND DISCUSSION

100 pathological and diagnosed samples were collected by specialist doctors from patients infected with skin fungi from different areas of the body from hospitals and some private clinics for the period between October 2023 and May 2024 for age groups between (1-70) years.

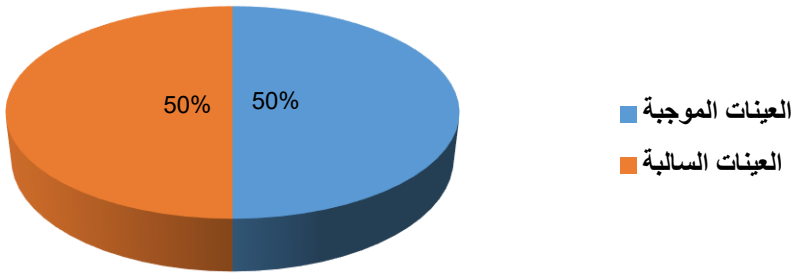

Figure (1): Represents the percentages of positive and negative samples for microscopic and cultural examinations.

The results showed that the most common type of skin fungus isolated during the study was Trichophyton mentagrophytes with 8 isolates and (16%) of the total number of positive samples for culture, which amounted to 50 isolates, followed by Microsporum canis with 6 isolates and (12%), while Candida albicans appeared in 5 isolates and (10%), followed by Candida tropicals with 4 isolates and (8%), Candida parapsilosis with 3 isolates (6%) and C. lipolatica with 2 isolates (4%), while T. verrucosum and Microsporum gypseum appeared with one isolate and (2%) each, and the molds Aspergillus niger and Aspergillus tubingenesis also appeared with (8%, 6%) respectively. Yeasts showed varying percentages of isolations, with Meyerozyme caribibica and Meyerozyme guilliermondi being the most isolated with 4 isolates each (8.8%), followed by Pichia guillierum with 3 isolates (6%).

Percentage	No	Type of parsite
16%	8	*Trichophyton mentagrophytes*
4%	2	*Trichophyton indotineae*
12%	6	*Microsporum canis*
10%	5	*Candida albicans*
8%	4	*Candida tropicals*
6%	3	*C. parapsilosis*
4%	2	*C. lipolatica*

2%	1	*C. kefyr*
2%	1	*C. rugose*
8%	4	*Aspergillus niger*
6%	3	*Aspergillus tubingenesis*
8%	4	*Meyerozyme caribibica*
8%	4	*Meyerozyme guilliermondi*
6%	3	*Pichia guillierum*
100%	50	Total

Trichophyton mentagrophytes colonies appeared within (7-10) days of incubation at 28°C and were characterized by being flat, white to cream in color, with a granular or powdery surface. Some colonies showed a central fold and the color of the back side was yellowish-brown or reddish-brown. Microscopic examination showed the presence of a number of large conidia and small conidia. The large conidia were cylindrical in shape with thin walls, with dimensions of 7-7 x 4-2 microns, containing 2-3 cells. As for the small conidia, they were spherical in shape, gathered in the form of clusters present along the fungal thread (Hypha), as shown in Figure (2).

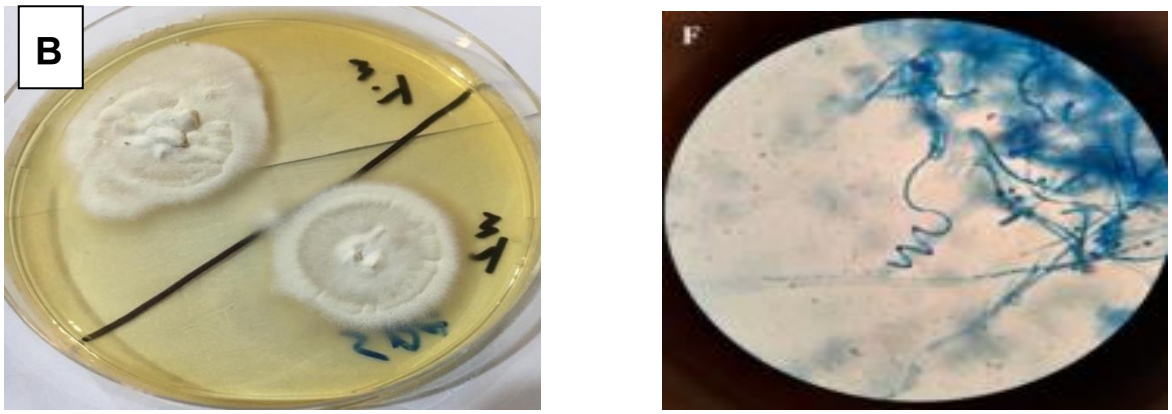

Figure (2): A- Trichophyton mentagrophytes under the microscope, B- Trichophyton mentagrophytes colonies on SDA agar

Trichophyton indotineae colonies appeared on SDA growth medium after (7-8 days) of incubation at a temperature of 28°C. The growing colony of the fungus is characterized by being fast-growing, flat granular, with a peripheral white color, a central beige to light brown color, and the reverse side of the colonies is light brown to yellowish. Microscopic examination under a power of (40 X) shows the hyphae in a twisted form with small and large microconidia, round and oval, with large conidia separated in a spindle shape. The conidia wall is smooth and contains a number of septa ranging between (3-4), while the small conidia appear spherical and are clustered in clusters along the fungal thread. Figure (3).

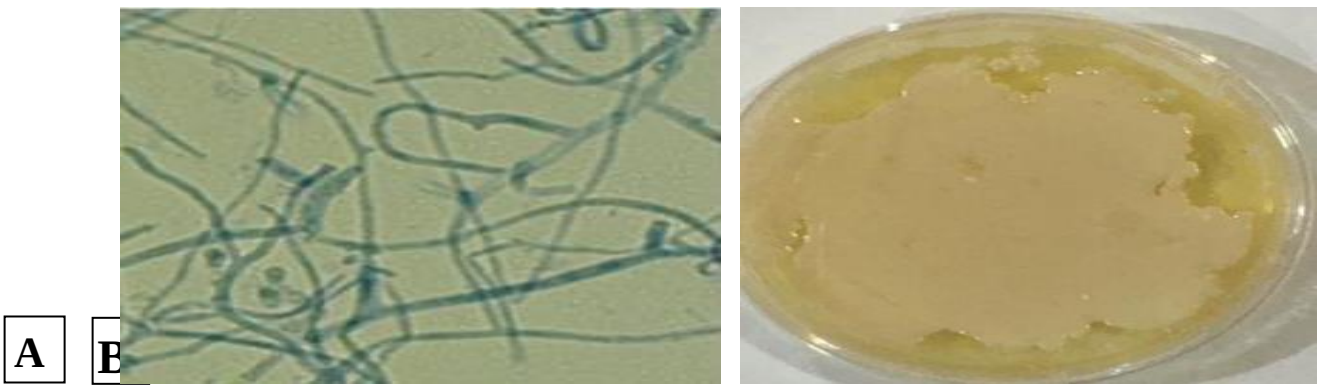

Figure (3): A- Trichophyton indotineae under the microscope B- Colonies of the fungus Trichophyton indotineae on SDA agar

Colonies of Microsporum canis, this fungus, growing on SDA medium at an incubation temperature of 25° appeared elevated above the surface of the medium and had radial grooves, and were yellowish white to light beige in color and had a cottony character with aerial hyphae protruding upwards microscopically and under force (X40). Large conidia were present in large numbers and had thickened walls, while small conidia were missing in most of the isolates. as shown in Figure (4).

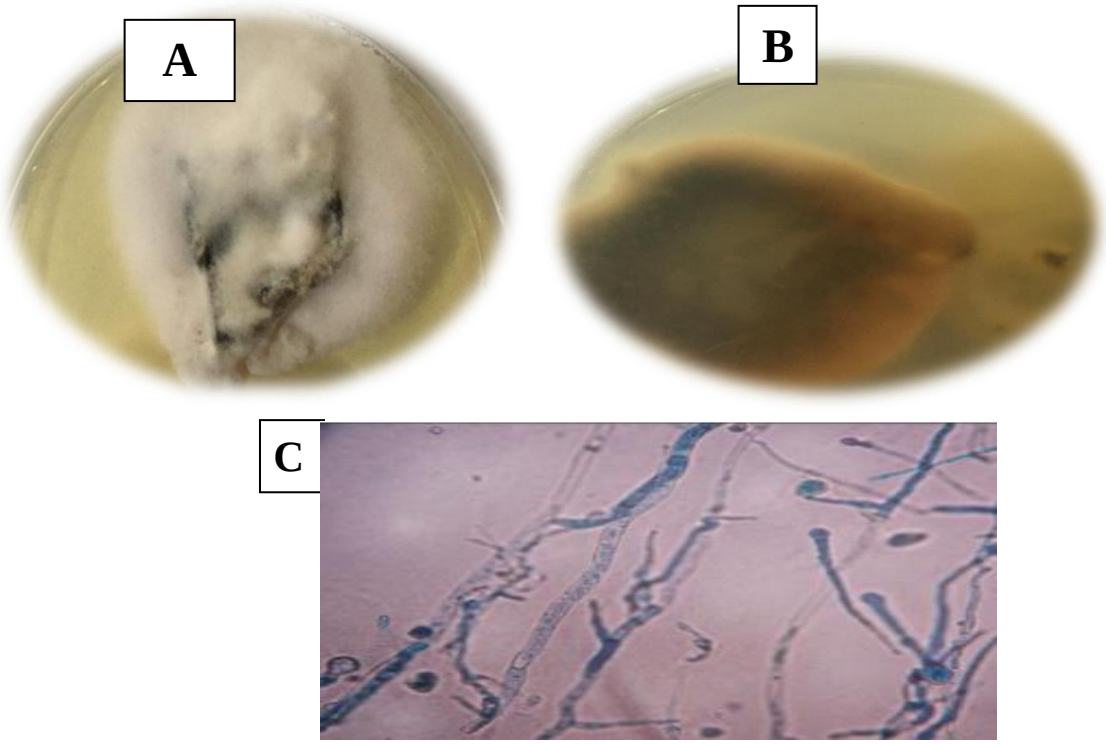

Figure (4): A- The reverse side of the colony on SDA agar medium, B- M. canis fungus colony on SDA agar medium C- M. canis fungus under the microscope (40 X)

Candida albicans fungal colonies appeared after one to two days of incubation at 28°C on SDA medium. They appeared smooth, slimy, creamy white, bimorphic, and had a yeasty odor. They appeared under the microscope as shown in Figure (5) in the form of oval cells when stained with lactophenol.

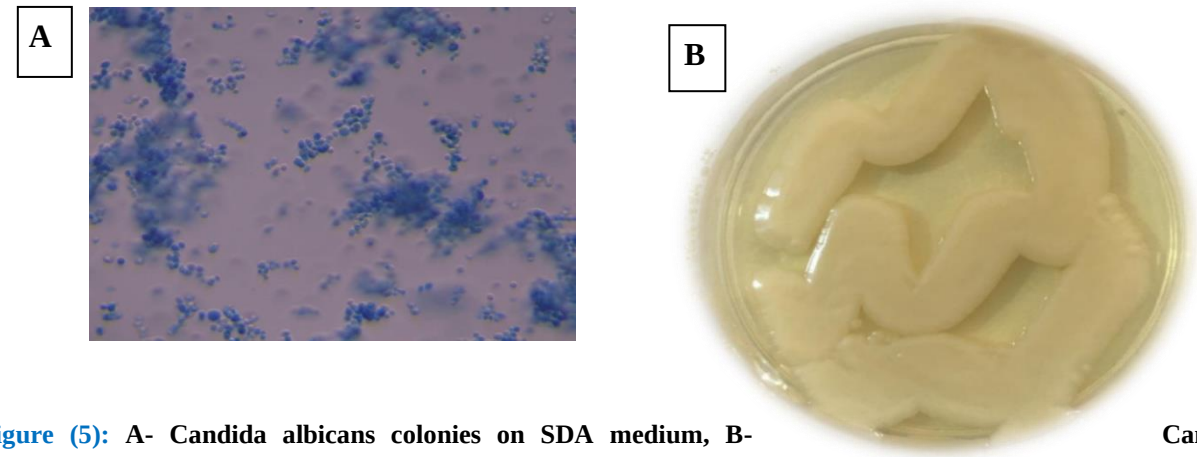

Figure (5): A- Candida albicans colonies on SDA medium, B- Candida albicans fungus under the microscope

Candida tropicals colonies appeared smooth, pale, cream-colored after 48 h of incubation at 30 m on SDA agar, as shown in Figure (6).

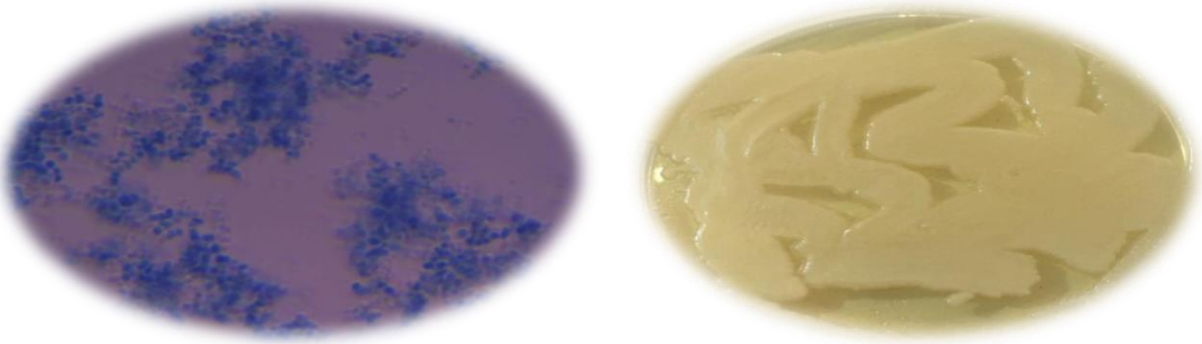

Figure (6): B- Candida tropicalis under the microscope, A- Candida tropicals colonies on SDA agar medium

Candida parapsilosis forms creamy white, smooth with focal wrinkles, and the colony reflectance is light yellow. C. parapsilosis culture is represented by yeast-like cells with round or cylindrical yeast cells with active budding, which is consistent with (Ge et al., 2019), as shown in Figure (7).

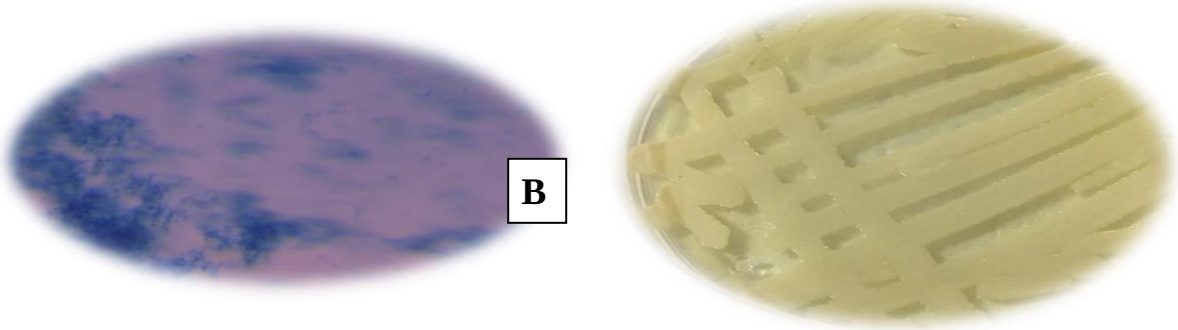

Figure (7): A- Candida parapsilosis under the microscope, B- Candida parapsilosis colonies on SDA agar medium

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Candida. Lipolatica fungal colonies appeared on SDA agar at 25°C as pale white and pointed as shown in Figure (8).

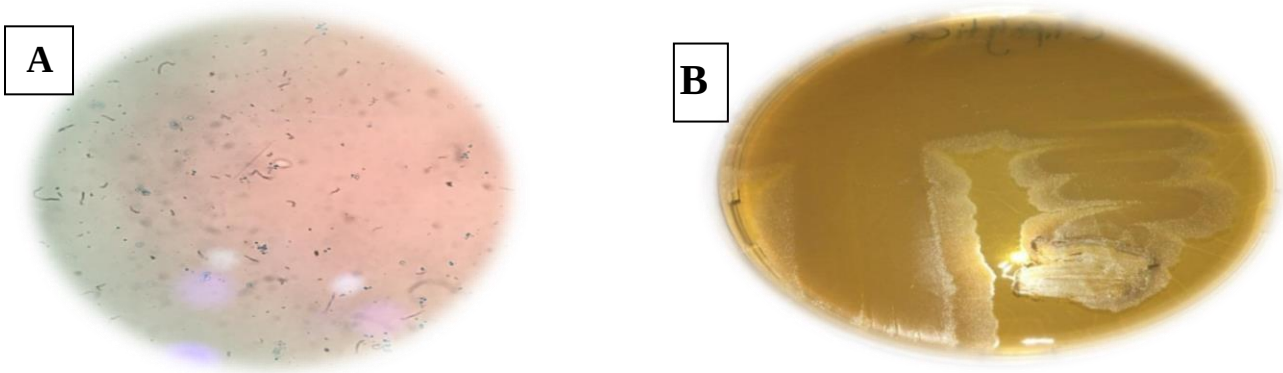

Figure (8): A- Candida Lipolatica under the microscope, B- Candida Lipolatica colonies on SDA agar medium

Colonies appeared on SDA medium incubated at 25-30°C as white, brown, mucous, smooth-surfaced colonies on the third day of incubation, as shown in Figure (9),

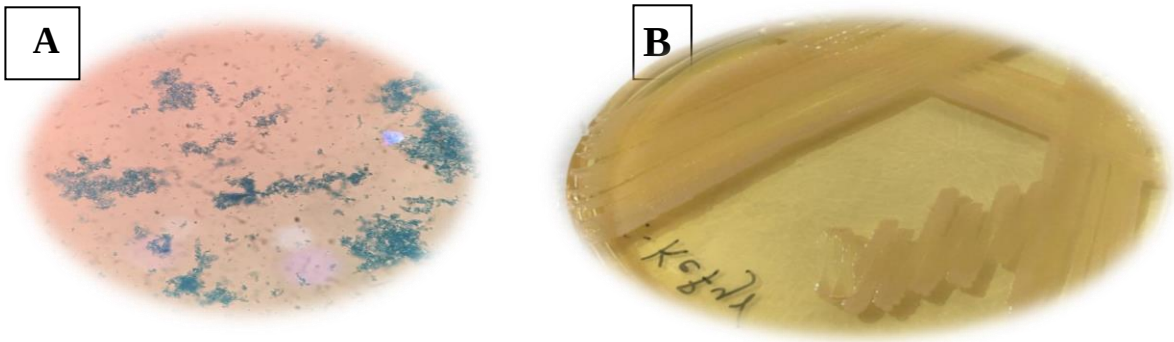

Figure (9): Candida Kefyr colonies on SDA agar medium

Candida rugosa colonies appeared white, wrinkled, and flat on SDA layers, shown in Figure (10).

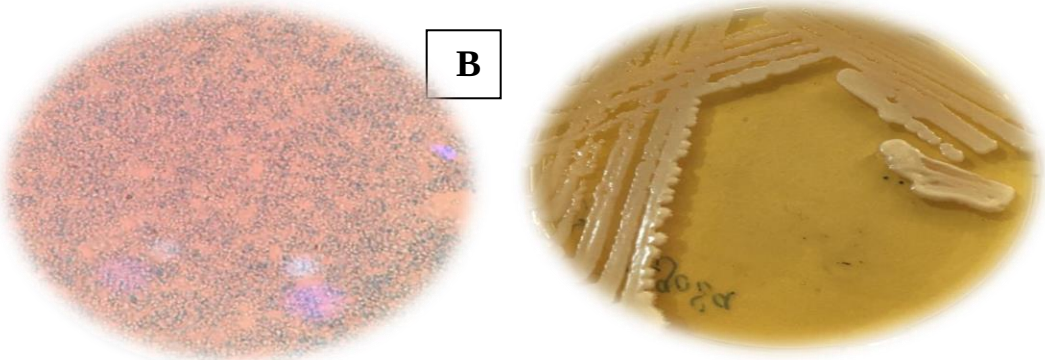

Figure (10): B- Candida rugose under the microscope, A- Candida rugose colonies on SDA agar medium

Colonies of Aspergillus spp. appeared on SDA medium after (5-7) days of incubation at 28°C. The colonies appeared as round, convex, with regular edges and white in color. At the beginning of their growth, the colonies appeared as cottony, resembling grains of sand, then turned black with brown edges. They appeared under the microscope as hyphae with divided conidia ending in round vesicles arranged on phloems in a radial appearance, as shown in Figure (11).

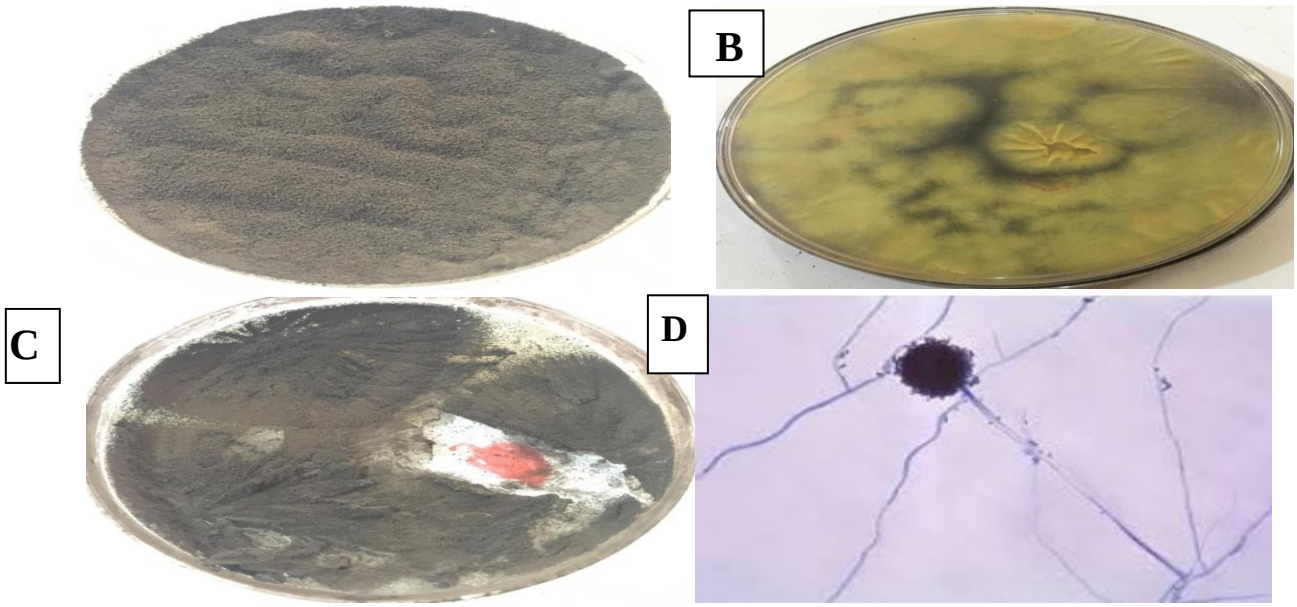

Figure (11): A, B- Aspergillus niger colonies on SDA agar medium,C- Aspergillus tubingensis colonies on SDA agar medium,D- Aspergillus fungus under the microscope (40 X).

Meyerozyma caribbica and M. guilliermondii show no differences in standard fermentation and growth tests. M. caribbica forms ascospores that are spherical in shape, unlike the cap-shaped ascospores of M. guilliermondii, but both species reproduce poorly, and separation of species on this criterion is not feasible. Therefore, it is recommended that the two species be separated by differences in rRNA gene sequences or by DNA recombination experiments. Fungal colonies appeared after 1–2 days of incubation at 28°C on SDA medium and were smooth, sticky, and creamy white in color, as shown in Figure (12)(13).

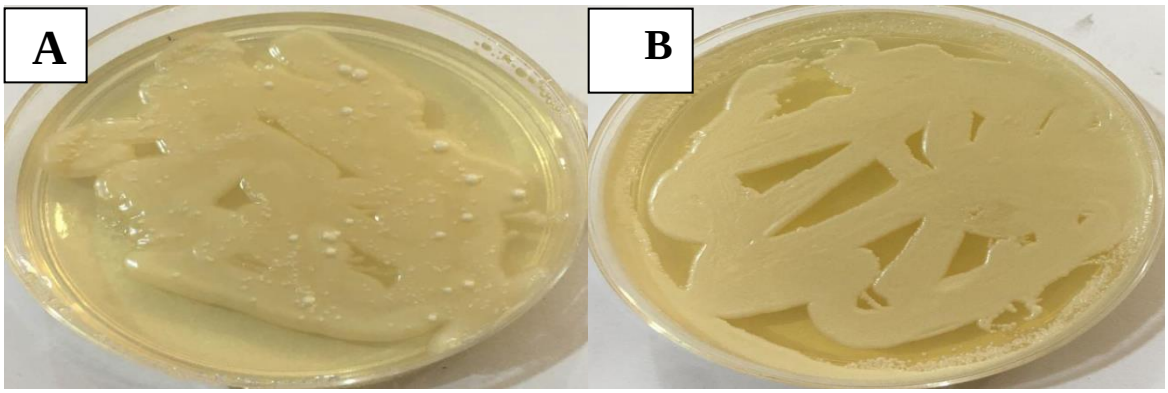

Figure (12): A- Meyerozyma caribbica colonies on SDA agar medium, B- M. guilliermondii colonies on SDA agar medium

Pichia guilliermondii is a teleomorph, forming cap-shaped, hemispherical or round ascospores during sexual reproduction. Its colonies appear flat, moist, smooth, creamy to yellow on SDA agar medium as shown in Figure (13),

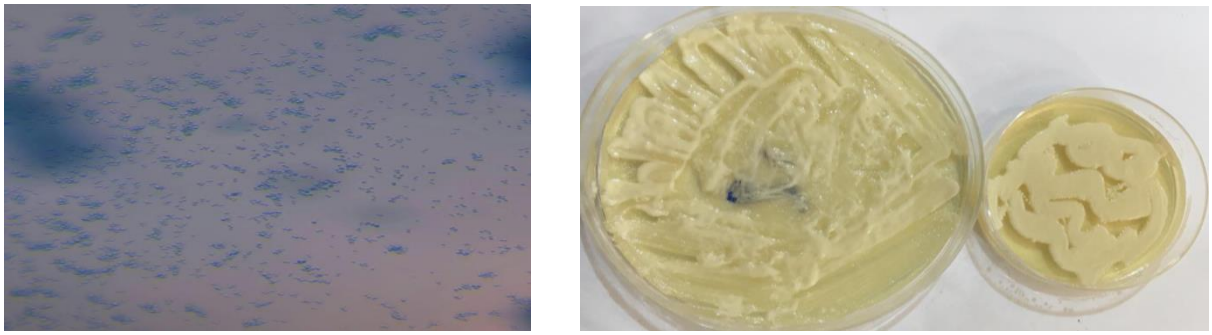

Figure (13): A- Under the microscope, B- Pichia guilliermondii colonies on SDA agar medium

Distribution of infection according to body areas

The results of the current study showed a variation in the number of swabs taken from patients infected with skin fungi according to the areas from which they were taken, where the largest number of swabs was from the foot at (17%), followed by the face and head at (16%, 15%) respectively, while the lowest number of swabs was from the legs at (7%). The study also showed a variation in the number of positive samples, where the largest number of positive fungal samples was taken from the head at (20%), followed by the fingers and between the toes at (16%, 14%) respectively, while the lowest percentage of positive samples was taken from the thigh area at (8%) as shown in Table (2).

Table (2): Distribution of skin fungal isolates according to the areas taken from them on the human body

%	Positive result	%	No	Isolates area
16%	8	10%	10	Fingers
20%	10	15%	15	Head
10%	5	13%	13	Hand
10%	5	17%	17	foot
12%	6	7%	7	legs
8%	4	10%	10	thigh
10%	5	16%	16	face
14%	7	12%	12	toes
100%	50	100%	100	Total

The results of the current study showed that the incidence rate varies according to the body regions, as it was found that Tinea capitis topped the incidence rate with 20%, as the number of patients reached 10. This result is consistent with what was reached by (14, 15), as the incidence of tinea capitis was ranked first among the total infections with a rate of 33.33%, 27.2% respectively, and it also agreed with what was reached by (16, 17), as they found that tinea capitis is the most affected area, as they explained that one of the main causes of head infections is indirect hair infection through the use of personal items such as hats and clothes, in addition to the fact that frequenting barbershops is one of the causes of head infections. In addition, the infection is transmitted by children in abundance due to mixing and lack of attention to their general hygiene. The second place was occupied by Tinea manum with a rate of 16% and the number of patients was 8 patients. These results do not agree with what was reached by (14), as tinea manum recorded the lowest infection rate of 2.4% with 3 infections out of the total number of infections. The difference in percentages is due to the size of the isolated sample and the nature of the patients' work.

Tinea manum was recorded at a rate of (10%), as tinea manum is mainly spread among people whose hands are immersed in water for a long time, such as housewives and cooks, as well as people who deal with fish or vegetables (18). Sometimes, tinea manum infection may be associated with infection of the feet as well, and tinea manum infection usually

occurs in only one hand (19). Athlete's foot is a problem in societies due to its contagious and recurring infection, as the absence of sebaceous glands under the foot and their ability to secrete several enzymes such as serine proteases, metalloproteases, keratinases, cysteine dioxygenase, are considered among the most important factors that help fungi cause athlete's foot infection, and it is more common among some professionals who always wear shoes, such as soldiers(20). Athlete's foot infection is widespread in swimming pools between April and September, especially in the spring and summer, when fungal infections are widespread (21). Climatic conditions such as heat and humidity are factors that help in causing foot infection, in addition to constant sweating of the feet and wearing shoes and socks constantly. Also, a lack of immunity in the body is one of the factors that help in causing foot infection (22). The percentage of tinea pedis reached (14%). The reason for the occurrence of tinea pedis is due to the ability of skin fungi to secrete the enzyme phospholipase and lipase, which play an important role in causing the infection. We note in the results that the percentages are close between tinea pedis and tinea pedis. We also note that the percentage of tinea pedis is equal to the percentage of tinea of the hand. This may be due to the transmission of infection from the foot to the fingers (23). Also, raising pets in homes and other animals such as cats contributes to the occurrence of fungal infections in the body (24).

4. CONCLUSION

The study concluded that the most common type of skin fungus isolated during the study was *Trichophyton mentagrophytes*, followed by *Microsporum canis*. The study revealed a variance in the quantity of positive samples, with the highest number of positive fungal samples obtained from the head, followed by the fingers and interdigital spaces, while the thigh area exhibited the lowest percentage of positive samples.

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