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Prevalence of Cutaneous mycosis in Baqubah City for the period from December 2021 to April 2022

Hussein Sami Ismael Al-Mayyahi*[1], Anaam Fuad Hussain [2] and Hayder Jasim Mohammed[2]

[1]Department of Biology – College of science – University of Diyala
[2]Department of Biotechnology – College of science – University of Diyala

hhssh23@yahoo.com

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Abstract

The current study aimed for isolation and identification of fungi from tinea types in patients who lived in Baqubah City- Diyala province, detect which clinical type of disease is the prevalent among the patients with their etiologic agents and detect the effective antifungals against isolated fungi. Eighty samples of onychomycosis swabs, nail clippings, skin fragments and hair were collected from sixty-five patients (some patients took more than one sample from them) from December 2021 to April 2022. Through direct microscopy and after culturing in the culture media, results showed that the total positive fungal isolates were 65 (81.25%) including dermatophytes 49 (75.38%) and opportunistic yeast fungi 16 (24.61%). Tinea corporis was the most common type of dermatophytosis in that the prevalence of dermatophytosis in males was higher than in females. The effect of terbinafine, itraconazole, fluconazole and nystatin were applied on fungal growth by well diffusion method and results concluded that terbinafine was more powerful and active against dermatophytes.

Keywords: Fungal skin infections, dermatophytes, opportunistic fungi, antifungals

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انتشار الإصابات الجلدية الفطرية في مدينة بعقوبة في الفترة من كانون الاول2021 الى نيسان 2022

حسين سامي اسماعيل[1]، انعام فؤاد حسين[2] وحيدر جاسم محمد[2]

[1]قسم علوم الحياة – كلية العلوم – جامعة ديالى
[2]قسم التقانة الاحيائية – كلية العلوم – جامعة ديالى

الخلاصة

إستهدفت الدراسة الحالية عزل وتشخيص الفطريات من أنواع السعفة لدى المرضى المصابين الذين يعيشون في مدينة بعقوبة - محافظة ديالى، والكشف عن النمط السريري السائد بين المرضى والنوع الفطري المسبب إضافة الى دراسة فاعلية مضادات الحياة تجاه الفطريات المسببة للمرض. جمعت ثمانين عينة (أشتملت على مسحة الفطريات، قطع من الأظافر، أجزاء من الشعر وبثور جلدية) من خمسة وستون مريضًا (بعض المرضى أخذت أكثر من عينة) للمدة من كانون الأول 2021 إلى نيسان 2022. من خلال الفحص المجهري المباشر وبعد الزرع على الأوساط الزرعية الخاصة. أظهرت النتائج أن مجموع العزلات الفطرية الموجبة 65 عزلة (81.25٪) متضمنة الفطريات الجلدية 49 (75.38٪) وفطر الخميرة الانتهازية 16 (24.61٪). كانت السعفة الجسدية هي الأكثر شيوعًا: حيث كان انتشار المرض عند الذكور أعلى مماهو في الإناث. تم دراسة تأثير فاعلية مضادات الفطريات الفلوكونازول والاتراكونازول والنستاتين والتربينافين بطريقة الانتشار من الحفر حيث أظهرت النتائج ان التربينافين كان الأكثر فاعلية ضد الفطريات الممرضة.

الكلمات المفتاحية: أمراض الجلد الفطرية، فطريات جلدية، فطريات إنتهازية، مضادات فطرية

Introduction

Skin, the biggest organ in the human body is home to many beneficial microbes and acts as a physical barrier to keep diseases out. When the barrier is breached or when the equilibrium between commensals and pathogens is upset, skin disease or even systemic disease can result [1]. Additionally, regional changes in skin's pH, sebaceous gland density, humidity, and temperature provide various ecological niches. Where, viruses , bacteria, fungi, archaea, and mites can be thriving [2]. However, This study focused in the infections of pathogenic fungi, including the of the skin and its appendages, such as hair and nails, are common in all regions of the world [3].

Dermatophytosis is the infection of keratinized tissue including hair, nails and the stratum corneum of skin; dermatophytic fungi have a unique interaction with the immune system that occur more frequent

in people with impaired cell mediated immunity [4][5]. The different dermatophytosis conditions also known as ringworm, are named according to the site [6], these infections include tinea capitis is the predominating fungal disease specially in male children (2-10) years old , tinea manuum ,tinea barbae ,tinea faciei ,tinea cruris ,tinea pedis , tinea unguium and tinea corporis [7][8]. Dermatophytoses are caused by species of three genera including *Epidermophyton floccosum, Microsporum* and *Trichophyton* [9][10]. Genera that caused opportunistic infections includes filamentous fungi (such as *Aspergillus, Penicillium, Mucor* and *Rhizopus*) and yeasts such as *Candida* and *Rhodotorula* [11].

Despite the fact that a variety of antifungal medications are available for therapeutic usage, these only affect a small number of cellular targets and fungi may develop a tolerance to or resistance to these medications, in addition to that the cost of fungi infections from an economic perspective is still too great [12]. Various factors such as the fungi static nature of,most drugs, wrong antifungal usage (under circumstances where the causative agent is known), Fungal resistance is influenced by low antifungal dosage, prolonged therapy, medication interactions, and therapy costs [13]. Human fungal infections are becoming more commonly, especially in immunocompromised people had led to the fact that these diseases are a global public health problem and still one of the most infections in Baqubah city, Diyala Province. The outcome of the illness, which can range from minor cutaneous or subcutaneous infections to invasive, widespread, and potentially fatal infections, is determined by the immunological health of the host. Therefore, The study aimed for isolation and identification of fungi from tinea types in patients lived in Baqubah City- Diyala province, detect which are prevalent type among the patients, with their etiologic agents and determine the effective antifungals against fungi.

Materials and Methods

Sampling: Eighty samples of onychomycosis swab, nail clippings, hair and skin fragments were collected from sixty five patients (for some patients, with many samples) who attended to Baqubah Teaching Hospital and outpatients visitors to the private clinic of professor doctor Khudhair Khalaf Al-Kayali, Diyala province from December 2021 to ,April 2022. The samples came from people of various ages, ranging from (7 to 60) years old. Samples included both genders (males 52 and females 13).

Mycological Investigation: This was carried out by dividing the samples into two parts for the following:

Direct Examination of fungi: Samples of nail clippings, hair samples and skin fragments were put under direct inspection by being placed on a clean glass slide. and drops of 10% KOH and 36%DMSO were added to digest proteinaceous debris in the samples for making the visibility of fungal elements clear like spores, mycelium, and budding or pseudohyphae (yeast), and covering with slide, then placed over the specimen. After that, the slide gently warmed with a burner's flame or left in the lab temperature for five minutes while being checked for fungal hyphae [14][15].

Cultural Examination (Culture of specimen): Samples of nail clippings, hair samples, skin fragments, onychomycosis, folliculitis swabs and boil swabs were cultured on Sabouraud Dextrose Agar (SDA), plates at pH 5.6 with addition of chloramphenicol 250 mg to make the medium selective to prevent the growth of saprotrophic fungal contamination. The plates were incubated in triplicate. at 35 ± ºC for 10 --14 days, with daily examination and observation.

A: Identification of dermatophytes: The following characteristics were taken into account of filamentous fungi: colony morphology (consistency and color), reverse color (color, changed with age), microscopic characteristics (arrangement, shape and conidial ontogeny, their size, micro conidia and macro conidia) [14[15].

The following identification of the most common dermatophytes species was determined by tests, and they include (*T. rubrum , T. mentagrophyte, and M. canis*)

1. Growth on Potato Dextrose Agar (PDA) Medium

This test was used to differentiate between (*T. rubrum*, and *T. mentagrophytes*) according to the ability of production red color on the reverse side of plates with PDA medium. From the colony's edge, fungus inoculum was removed and placed in the middle of plates and then the plates were incubated at 28ºC±2 and after 14 days, looked at the media's color by observing it. [16].

2. Growth at Temperature 37ºC

This test was used to differentiate between a few species that were connected to the genus *Trichophyton* by their ability to grow at temperature 37 ºC such as (*T. mentagrophytes*) and (*T. rubrum)* which unable to grow at this temperature [15].

B: Identification of yeasts: SDA was used for primary isolation of *Candida spp* and other yeasts, it had a low pH that allows *Candida* to growth and inhibits the growth of most bacteria but not all. Therefore,

supplementation of SDA, with of chloramphenicol 250 mg made the medium selective to prevent the growth of saprotrophic fungal contamination. Parafilm was used to stretch the plates to prevent contamination, and the plates were kept in the refrigerator to preserve the culture [14]. The isolates had a stain applied by Lactophenol cotton blue to determine how they react to stains, as well as their organization to shapes and yeast budding type [17]. India ink was used to detect the capsulated yeast [15].

For differentiate *C .albicans* from other species, according to Forbes *et al.* [18], the quick nutritional diagnostic test was evaluated on germ tube formation. stop other yeast species from forming germ tubes, The cells were incubated in 0.5 ml of human serum at 37°C for two hours, but no more than three hours. On a slide was inserted a drop of the incubated serum, subsequently covered with a cover-slip, and the presence of germ tubes was checked under a microscope.

Species identification of *Candida* was made by CHROM, agar Candida: By inoculating a loop full of culture, pure isolates of *Candida* were revived from SDA in to 10 ml sterile Nutrient broth that incubated at 37º for 3 days cultures were cautiously inoculated on the surface of the CHROM agar media after 72 hours of incubation. by streaking a loop full of culture and incubated at37º for 72-96hrs with the plate facing up right. After 72-96 hours of incubation all plates removed from the incubator and the results were recorded. When compared to normal color images provided by the manufacturer and also those published by Odds and Bernaerts, yeast colonies were initially identified by colonial color [19].

Antifungal sensitivity Test: The experiment of commercially available samples of antifungal drugs used in this study (Terbinafine, Itraconazole, fluconazole and Nystatin) were done to detect fungal sensitivity percentage of yeast and dermatophytes species.

1. Fungal Inoculum Preparation: Fungal inoculum of filamentous fungi (dermatophytes) was prepared by taking the fungal growth from the surface of the colony that aged 5-7 days by metal Needle, then it was transferred to test tubes contains 5ml of normal saline (0.85% NaCl) containing 0.05% Tween 80 for 5min. and the hyphal pieces were gently filtered out of the conidia suspension after it had been gently probed with a pipette tip. The tube was shacked well by vortex. Finally standard solution of dermatophytes was prepared (to contain 1x 10^6 spores ml^{-1}) by the use of haemocytometer to count fungal conidia [20]. While preparation of yeast suspension was performed after checking the purity of tested yeast; the isolates with 18-24 hours were transferred to 5ml physiological solution then mixed well to prepare a homogenous

yeast suspension with a turbidity equivalent to no. 2 McFarland respectively by using a Densichek instrument [21].

2. Antifungal Agent sensitivity

Each antifungal was prepared as an initial concentration" working stock solution" of 10,000 Mg ml^{-1} [22] as follow:

1. One hundred mg for each drug was added to 10 ml of dimethyl sulfoxide and shacked vigorously by vortex. This gave a final concentration of (10,000 µg ml-1 (then it was transferred to sterilized vials.

2. The stock solution was left at room completely for 30 min, this permits self-sterilization of the drugs by dimethyl sulfoxide. The stock solution was kept fridge until used.

3. All plates were inoculated at the same time and left for one hour, and then pits were made (for each drug) by cork borer to give 5ml diameter in culture media, then 50 µL of SDA were put in each pit base to prevent the leak of antifungal.

4. Finally 0.1 ml from each antifungal was applied in pits, and all plates were incubated at 28±2ºC. The plates were daily examined until the recording of results.

Results and Discussion

Sampling and Isolation of Fungi

Through direct microscopy and after culturing in the culture media. The results showed that the total positive fungal isolates were 65 (81.25%) include dermatophytes 49 (75.38%) and opportunistic yeast fungi 16 (24.61%). The results presented that only 15 (18.75%) specimens were negative, both in direct microscopic inspection and in culturing.

A considerable number of the patients was 65(81.25%). The patients were clinically diagnosed and mycologically confirmed (figure 1) to have tinea corporis. (21cases ;32.3%) presented with pruritic, characteristic annular progressive scaly plaque with aburning sensation and healed center; tinea capitis (14cases ;21.53 %) presented with hair loss with scaling; tinea unguium (7cases;10.76 %)presented changes in the appearance of nail; tinea pedis (4 cases;6.15 %) presented with moist peeling irritable skin between the toes; tinea cruris.(2 cases; 3.07%) manifested with erythematous spots in the groin that

had a crisp border and pruritus; and tinea mannum (one case only; 1.53%) presented with red and scaly areas. Tinea corporis was the most common type of dermatophytosis followed by tinea capitis and that was in agreement with Naseri *et al* [23] in the males were more likely than females to have dermatophytosis. However, table (1) shows the number of specimens according to the clinical types of dermatomycoses.

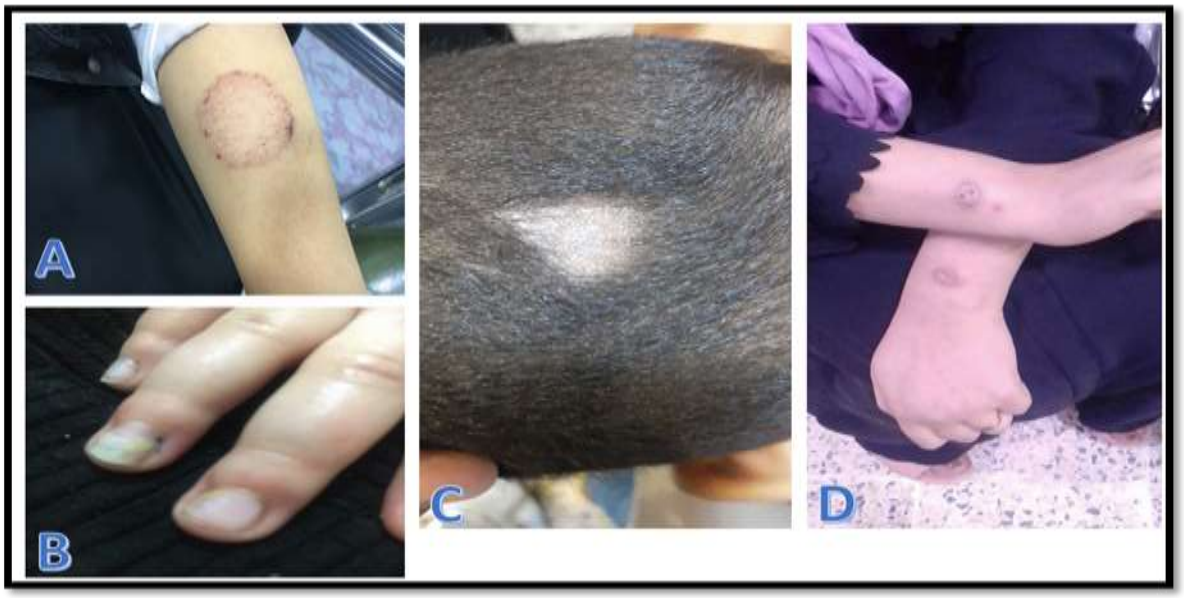

Figure 1: Clinical types of dermatomycosis: A: Tinea corporis circular:erythematous scaly lesions with advancing:margin on the arm; B: Tinea unguium; changes in the appearance of nail; C: Tinea capitis showing circular lesions with scaling; D: Tinea mannum showing red and scaly areas.

Table 1: Distribution of clinical types of dermatomycosis in relation to the age and gender of patients

Types of infection	Groups of Age (Years old)			Gender		Total
	7-20	21-40	41-60	Male	Female	
Tinea corporis	2	15	4	21	-	21(32.3 %)
Tinea capitis	12	2	-	14	-	14 (21.53 %)
Tinea unguium	1	6	-	2	5	7 (10.76 %)
Tinea pedis	-	4	-	4	-	4 (6.15 %)
Tinea cruris	-	2	-	-	2	2 (3.07 %)
Tinea mannum	-	1	-	-	1	1 (1.53%)
Onychomycosis	2	2	12	11	5	16 (24.61%)

Identification of Fungi

Sixty five samples of primary fungus isolation and identification from clinical samples were found using culture on SDA. All cases were positive in both the culture and microscopic examination. However, the culture was better confirming accuracy than the microscopic mount for identification the fungal genera. The specificity and sensitivity of the 10%KOH and 36% DMSO mount in comparison to the results of the reference standard culture examination, 100% was calculated. When using another two parameters; growth at temperature 37 ºC in SDA medium and culture on PDA medium in addition to indirect microscopic examination using Lactophenol cotton blue staining, four species of dermatophytes isolates were identified in 49 isolates (Table2): *Epidermophyton floccosum* (anthropophilic) 20 isolates, *Microsporum canis* (zoophilic) 13, *Trichophyton mentagrophytes* (zoophilic) 6 and 2 isolates of *Trichophyton rubrum* (anthropophilic).

The laboratory characteristics of dermatophytes were in agreement with many previous studies [11][24][25]. Colonies of *E. floccosum* on SDA after two weeks were greenish brown to khaki-coloured with a suede-like surface with 8 cm in diameter, old cultures developed white pleomorphic tufts of mycelium; microscopic feature showed smooth, thin walled macroconidia produced in clusters growing directly from the hyphae, microconidia were not formed. The colonies of *M.canis* grew on PDA with diameter 7.4 cm after 12 days of incubation at 32ºC, seemed to be white to pale yellow in color with septate macroconidia. The colonies of *T. mentagrophytes* and *T. rubrum* grew rapidly on SDA and PDA respectively with diameter 8 and 7 cm respectively after 10 days of incubation at 32ºC, colonial morphology of *T. mentagrophytes* characterized as diffused granular, flat and powdery and cylindrical macroconidia with 2-5 cells and smooth wall and few in number, while micro conidia were spherical in shape and more in number. While colonial morphology of *T.rubrum* was variable, most cultures were white and floccose or pinkish with red or brown pigments on the reverse side, microconidia were clavate and numerous, in addition plenty of macroconidia were observed as a rare characteristic of this species which is in agreement with Brasch [26] who observed that in *T. rubrum* Var. raubitschekii.

The results of biochemical and physiological characteristics to species are shown to help with dermatophytes diagnosis in table (3). Results showed that *T. rubrum* has the capacity to red pigments to be produced on PDA medium, while *T. mentagrophytes* and *M. canis* lack this character. *M. canis* and *T. mentagrophytes* has the able to grow at 37ºC, while *T. rubrum* lost this character. The ability of growth of *T. mentagrophytes* at 37 ^{0}C, while *T. rubrum* cannot, provided physiological support for the

distinction between the two species [27]. All results were agreed to with Hasan [28]. Figure (2); shows macroscopic and microscopic appearance of *Epidermophyton floccosum* which was the most prevalent cause of dermatophytes in clinical types of skin infections. Figure (3); shows macroscopic and microscopic appearance of *T. rubrum* which was the least prevalent cause of dermatophytes in clinical types of skin infections. Dermatophytes are very common; they can affect both healthy and immunocompromised people, who participate in sports with contact, such as wrestling, awareness of and indifference to personal hygiene, sharing of private goods, exposure to dirt, and even animal contact. However, they outbreaks of diseases can happen in homes, schools, and other institutional settings; they are more frequently observed in hot, humid surroundings [29] [30].

Sixteen isolates of the yeasts were identified directly using 10%KOH and 36%DMSO and indirectly in primary isolation and identification of fungi from the clinical samples using SDA medium in addition to indirect microscopical test using Lactophenol cotton blue stain and India ink ; these isolates were 11 isolates of *Candida spp* and 5 isolates of *Rhodotorula mucillaginosa*. Up on morphological culture on Sabouraud dextrose agar SDA medium,the colonies of *Candida* isolates appeared as white to cream, curved, round, soft and smooth to wrinkled, with characteristic yeast odor, it was grew matured and rapidly in two days. While the colony of *Rhodotorula mucillaginosa* was characterized with coral pink, usually smooth, moist to mucoid, yeast-like colonies. Under microscopic examination using Lactophenol cotton blue, the blastoconidia of *Candida* were clear. When using India ink, spherical to elongate budding yeast-like cells or blast conidia, capsulated cell was clear and this was a confirmatory examination of *R. mucillaginosa*.

The germ tubes were formed within two hours of incubation, other yeasts often do not form germ tubes over this 3-hour period as time clears [31], these results were in agreed with Kidd and Hussain [11][24]. However, this is not a specific diagnosing feature of *C. albicans* to differentiate it from other type of *Candida*. Actually, the obtained results of evaluating CHROM agar Candida (CAC) as a source medium for identification *C. albicans* were confirmed by an earlier study by Mahmoudabad and Tamura [19][32] who found that *C .albicans* appeared in green color, these investigation were in agreement with other previous studies [11] [24]. The resulted different colors may be depending on the reaction between enzyme type released from Candida and the chromogenic mix (a component in this medium), the reactions produced during incubation are revealed by spontaneous color changes in the organism [33]. Figures (4) and (5) show the macroscopic and microscopic appearance of *C. albicans* and *R. mucillaginosa.*

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Table 2: Isolated species of dermatophytes and opportunistic fungi in relation to clinical types of dermatomycosis

Clinical type of infection	Species of Fungi (No. / percentage)					
	E. floccosum	M.canis	T. rubrum	T.mentagrophyte	C.albicans	R.mucillaginosa
Tinea corporis	9 (18.36)	4 (8.16)	2 (4.08)	4 (8.16)	2 (4.08)	-
Tinea capitis	-	8 (16.32)	-	4 (8.16)	-	1 (2.04)
Tinea unguium	6 (12.24)	-	-	-	1 (2.04)	-
Tinea pedis	2 (4.08)	1 (2.04)	-	-	1 (2.04)	-
Tinea cruris	2 (4.08)			-	-	1 (2.04)
Tinea mannum	1 (2.04)	-	-	-	-	-
Onychomycosis	-	-	-	-	7 (14.28)	3 (6.12)

Table 3: Differentiate among dermatophytes species according to some of physiological characteristics

Dermatophytes Species	Growth at 37 0C	Growth on PDA medium
Microsporum canis	+	-
Trichophyton rubrum	-	+
T.mentagrophytes	+	-

+: positive

-: negative

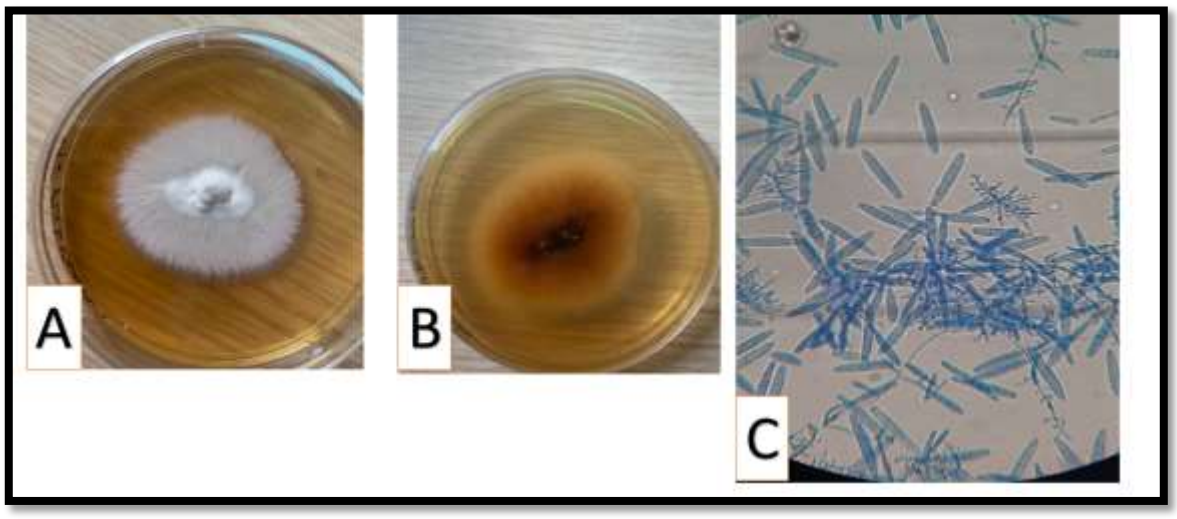

Figure 2: *Trichophyton rubrum* , A: macroscopic top view grown on PDA at 28±°C and pH 5.6 for 10 days of incubation, b-reversed view, C: microscopic appearance stained with Lactophenol cotton blue(40 X) showing clavate microconidia and plenty of macroconidia

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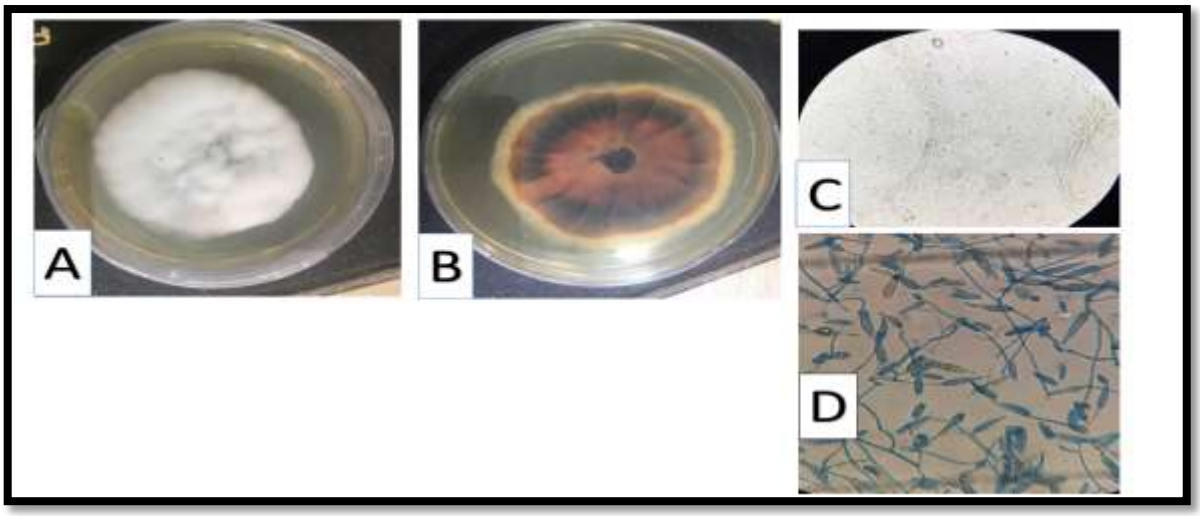

Figure 3: *Epidermophyton floccosum*, A: macroscopic top view grown on SDA at 28±2°C and pH 5.6 for 10 days of incubation, b-reversed view, C: microscopic appearance of 10%KOH and 40% DMSO mount of skin scrab, showing septate hyphae; the cuticle of the skin was destroyed, D: microscopic feature stained with Lactophenol cotton blue (40X) multiple smooth, club-shaped macroconidia

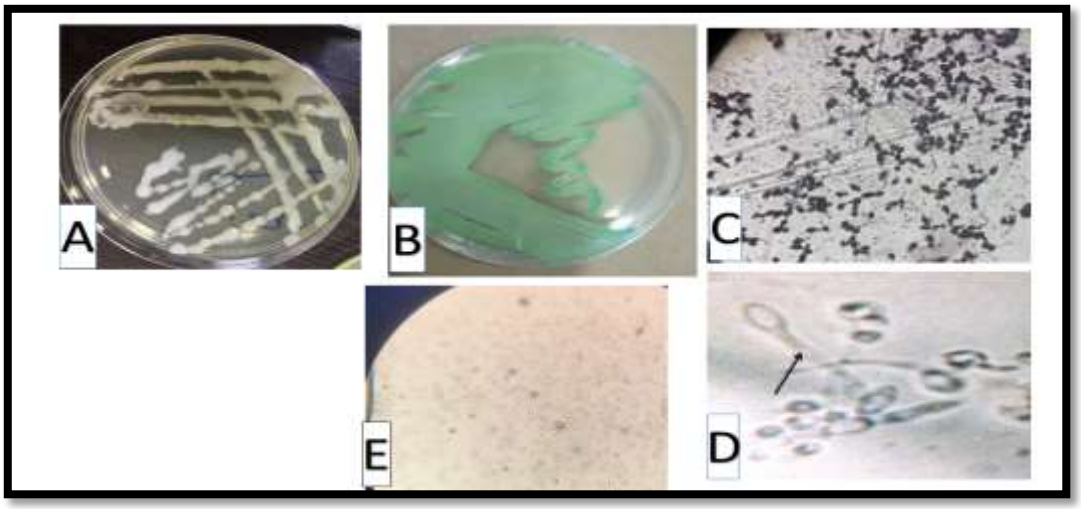

Figure 4: *Candida albicans;* A: grown on SDA at 35±2°C and pH 5.6 for 2 days of incubation, B: grown on Chrom Candida Agar at37°C after (2 days)of incubation, C: microscopic feature stained with Lactophenol cotton blue (40 X) showing budding blastospores, D: Germ tube formation (40X) when inoculated in serum after 2 hrs of incubation at 37ºC. E: microscopic appearance of 10%KOH/40%DMSO mount of skin scrab, showing, budding cell; the cuticle of the skin was destroyed.

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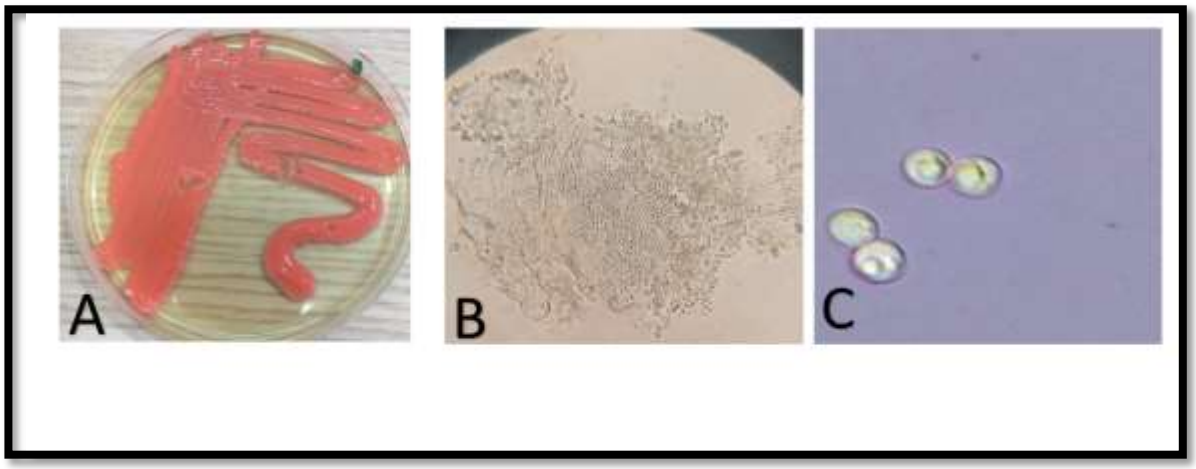

Figure 5: *Rhodotorula mucillaginosa* ; A: smooth, moist to mucoid colonies grown on SDA at 35±2°C and pH 5.6 for 2 days of incubation, B : microscopic appearance of 10% KOH/40% DMSO mount of head skin scrab, showing budding cell; the cuticle of the skin was destroyed, C: microscopic feature stained with India ink (40X) showing budding capsulated budding yeast

Activity of antifungal agents

The effect of four antifungal agents (Terbinafine, Itraconazole, Fluconazole and Nystatin) on fungal growth (for all fungal isolates) by well diffusion method were studied and the results were measured by observing the inhibition zones; the results illustrated in table (4), showed that all dermatophytes and yeast species in most isolates have the sensitivity toward terbinafine. This result was in agreement with Abid Ali [34] who concluded that terbinafine was characterized by interference with enzymatic activity of pathogenic fungi and with Hasan [28] who concluded that this drug is more powerful and active against dermatophytes. While the most isolates were resist to itraconazole. Fluconazole showed no action towards dermatophytes (Figure 6) with high effectiveness against *R. mucillaginosa*. The resistance toward antifungal may be because of abusing drugs which leads to increase resistance of patients [35], so clinicians should avoid the use of fluconazole for the dermatophytes sensitivity.

Nystatin appeared effectiveness against yeasts and anthropophilic dermatophytes, Goldstein and Beth [36] mentioned previously that nystatin is effective for cutaneous candidiasis but not for the treatment of dermatophytes, however it was effective for *T. rubrum* in present study; therefore, treatments with antifungals require a sensitivity test at laboratory of mycology to get the suitable antifungal and dose for the patients [37].

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Table 4: Distribution of antifungal susceptibility pattern of different dermatophytes and yeasts species

Fungal species	Antifungal drugs Resistance (No. (%))			
	Fluconazole	Itraconazole	Nystatin	Terbinafine
E. floccosum	20 (100)	20 (100)	2 (10)	0(0)
M. canis	13 (100)	12 (92.3)	13 (100)	2 (15.38)
T. mentagrophyte	8 (100)	6 (75)	8 (100)	1 (12.5)
T. rubrum	2 (100)	1(50)	0(0)	0(0)
C. albicans	9 (81.81)	11 (100)	0(0)	2 (18.18)
R. mucillaginosa	0(0)	5 (100)	2 (40)	0(0)

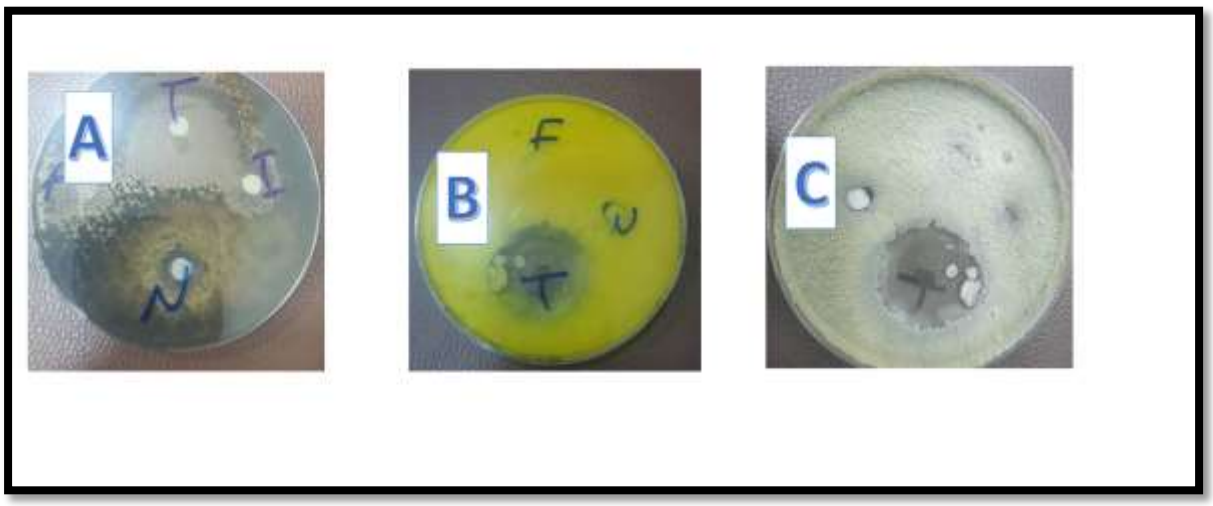

Figure 6: Effect of antifungal agents at concentration equal to 10 µg ml^{-1}against A*: E. floccosum*, B: *M. canis* and C: *T. mentagrophyte* on SDA medium at 32 ºC for 7-10 days

F: Fluconazole, I: Itraconazole, N: Nystatin, T: Terbinafine

Conclusion

Strengthening the immune system, self-cleaning, and knowing the frequency of illness possibly play a significant part in controlling infection and a highly effective scientific disciplines cooperation among dermatologists, mycologists and clinical laboratories is recommended.

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