Distribution of Dermatomycoses Fungi Among the Residents of Al - Khalis City: A Clinical and Phenotypic Study

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Abstract: Dermatomycoses are superficial fungal infections caused mainly by dermatophytes and, in some cases, by opportunistic fungi, particularly in susceptible individuals. This study aimed to investigate the clinical characteristics of cutaneous mycoses and identify the dermatophyte and opportunistic fungal species associated with these infections. A total of 120 clinical specimens, including nail clippings, onychomycosis samples, hair fragments, and skin scrapings, were collected from 100 patients with different forms of tinea infections in Al-Khalis city, Diyala Governorate, Iraq, during the period from May 2025 to February 2026. All specimens were examined by direct microscopy using 10% potassium hydroxide (KOH) and cultured on Sabouraud Dextrose Agar (SDA), followed by additional phenotypic identification procedures. The results showed that dermatomycoses were more frequently observed among females than males. Tinea manuum was the most prevalent clinical form, whereas tinea faciei was the least common. Fungal growth was obtained from 115 specimens (95.83%), including 90 dermatophyte isolates (78.26%) and 25 opportunistic fungal isolates (21.74%). Among dermatophytes, *Epidermophyton floccosum* was the predominant species (32 isolates), followed by *Trichophyton rubrum* (29 isolates), *Microsporum canis* (18 isolates), and *T. mentagrophytes* (11 isolates). Opportunistic fungi included *Candida albicans* (12 isolates), *Rhodotorula mucilaginosa* (4 isolates), *Cryptococcus neoformans*, *Aspergillus nidulans*, and *A. niger* (2 isolates each), as well as single isolates of *Rhizopus arrhizus*, *Penicillium* sp., and *Trichoderma longibrachiatum*. The isolation of *T. longibrachiatum* from tinea cruris represents an uncommon finding and highlights the emergence of less frequently reported fungal agents in superficial infections. These findings emphasize the importance of accurate laboratory identification combining microscopy and culture methods for effective diagnosis and epidemiological surveillance of dermatomycoses. Continuous monitoring and further molecular investigations are recommended to better understand the pathogenic potential and antifungal response of emerging fungal species.

1 INTRODUCTION

Fungi are considered non-photosynthetic organisms that obtain nutrients by absorbing preformed organic compounds from other organisms. The vast majority of fungi are considered saprophytic, which obtain their nutrients from decaying matter, on the other hand small proportion obtain nutrients from living hosts; therefore, they are classified as parasitic. In addition, fungi exhibit morphological forms such as unicellular forms (yeasts) or multicellular forms (molds). Fungi have significant beneficial impacts and detrimental roles in their interactions with humans [1]. Several fungal species have the ability to cause serious infections in humans, several of which may be life-threatening if they remain untreated. These include Aspergillosis, Candidiasis, Histoplasmosis, Mycetomas, Coccidioidomycosis, Cryptococcosis, and Paracoccidioidomycosis. Furthermore, the risk of these diseases is increased in persons with immunodeficiencies who are

particularly exposed to disease by genera such as Aspergillus, Candida, and Cryptococcus [2], [3]. Other fungal species are dermatophytic and keratinophilic, capable of infecting keratinized tissues in nails, hair, and skin and causing localized infections, including ringworm and athlete's foot [4]-[6].

Dermatophytes have evolved the capacity to utilize keratin as a nutrient source, a highly resistant structural protein that is largely inaccessible to most microorganisms. The fungi are capable of attacking nails, skin, and hair, where keratin is the major structural protein, resulting in a wide range of disease conditions. Dermatophytes infections routinely affect individuals who are otherwise healthy, but people with compromised immune systems are particularly susceptible and immunocompromised patients have been successfully treated with oral antifungals [6], [7]. Dermatophytoses are considered cutaneous infections which caused by dermatophytes that are characterized by their ability to invade and parasitize the non- living, keratinized layers [8]. They are classified as anthropophilic, zoophilic, or geophilic depending on whether their natural habitat is humans, animals, or soil [1] (Table 1).

Table 1: Grouping of dermatophytes on the basis of host preference and natural habitat [1].

Anthropophilic species	Zoophilic species	Geophilic species
Epidermatophyt one flocossum Trichophyton mentagrophytes T. rubrum T .tonsurans T. violaceum T. schoenleinii T. soudanense T. megninii T. concentricum Microsporum audouinii M. ferrugineum	T.mentagrophy tes (rodents, rabbits and cavies) T. erinacei T. verrucosum (cattle) T. equinum (horses) T. simii (monkey) M. canis (cats, dogs and horses) M. gallinae (poultry) M. nanum (pigs) M. persicolor (rodents and other small mammals)	M. gypseum M.vanbreuseghe mii M. racemosum

Normally, opportunistic mycoses are not capable of causing disease in healthy individuals, but they cause serious infections in immunocompromised individuals. These infections are commonly caused by genera such as Candida, Cryptococcus, Aspergillus, Mucor, Rhizopus, and Pneumocystis [9].

A common mistake that many clinicians make is to prescribe combination antifungal corticosteroid products (e.g., Cortisone) for the treatment of common fungal skin infections without confirming the diagnosis. This approach is not recommended due to the exacerbation of tinea infections caused by corticosteroid administration, which may contribute to treatment failure, especially when infections are due to Microsporum species [8], [10]. This study aimed to determine the clinical features of cutaneous mycoses that might affect patients who lived in Al-Khalis city, Diyala Governorate, using Sabouraud Agar (SDA), Potato Dextrose Agar (PDA), and CHROM Agar Candida (CAC) media, then isolation and identification of dermatophytes and opportunistic fungi.

2 METHODOLOGY

2.1 Sterilization Methods

Media and solutes were sterilized by autoclaving at 121 ºC, 15psi pressure for 15 minutes. All pieces of equipment's which are not affected by high temperatures were sterilized by electric oven at 180 ºC 1.5 hrs [11].

2.2 Preparation of Culture Media

2.2.1 Preparation of Ready to Use Media

Sabouraud's Dextrose Agar Medium (SDA), and Potato Dextrose Agar (PDA): These media were prepared according to manufacturer instructions, and used by supplementation of SDA with actidione10% (10 ml / L) to prevent the growth of environmental fungi and other contaminants.

2.2.2 CHROM Agar Candida (CAC)

CHROM Agar Candida (CAC). This medium was used for Candida species identification. CHROMagar candida was prepared according to Mahmoudabad et al [12] by adding preweighed amount 48 g/L in pre-sterilized distilled water, the media was simmered for five min and allowed to cool to a temperature below 50ºC before transferring 25 ml of media in to sterile 90 mm concord plastic petri dishes for solidifying with the partially opened lid. The plates were stored at 4ºC prior to use.

2.3 Preparation of Potassium-Hydroxide 10% Solution

This solution was prepared by dissolving 10 gm of KOH in 90 ml. of distilled water then adding 10 ml. of glycerol to prevent crystallization of solution and prevent specimen from dryness.

2.4 Tinea Diagnosis and Mycological Sampling

In this study, a total of one hundred twenty specimens of skin scrab, onychomycosis swab, hair fragments and nail clippings were collected from 100 individuals (with multiple samples obtained from some patients) were collected from both inpatients at Al-Khalis General Hospital and outpatients who attending a private dermatology clinic during the period that confined from May 2025 to February 2026; According to ethics book numbered (CEPEC/1378) clinical diagnosis of tinea type was performed according to Zafar et al [13] with the help of a dermatologist.

Mycological examination was made for the patients. The investigation was carried out by taking samples using sterilized instruments then transferred to fungal laboratory of fungi at the college of science / University of Diyala to direct exam and culturing.

2.5 Direct Examination

Samples of skin scrab and clippings of nails were subjected for direct exam as follow: sample was placed on clean glass slide and adding drops of KOH 10% then the sample was covered by cover slip. After that the slide left for 5 min in the lab before being examined microscopically for fungal elements, including hyphae and spores [14].

2.6 Indirect Examination (Culture of Specimen)

Specimens of nails and skin scales were cultured on (SDA) plates suplemented with actidione and adjusting Ph at 6.5, plate was incubated at 28 ±2 ºC for 10 -14 days to detect dermatophyte, other plate for 3-7 days to detect the opportunistic filametous fungi, the third plate was incubated at 35 ±2 ºC for two days to detect the yeasts, with daily examination and observation (each plate was triplicate).

Identification was based on several criteria, including morphology (color and consistency), reverse pigmentation (which changes with age), and microscopic characteristics (macro and micro conidia, arrangement, size, conidial ontogeny, and shape) [1], [8], [13]. Differential examination among common species of Trichophyton including T. mentagrophyte and T. rubrum and was conducted as bellow:

1) Growth on PDA Medium. This experiment, performed according to Milne [15], aimed to distinguish between dermatophyte species, including T. rubrum, Trichophyton mentagrophytes, and Microsporum canis based on their ability to produce red color on the reverse side of plates with PDA medium. Fungal inoculum was obtained from the colony's edge and placed in the middle of the plates. The plates were incubated at 28±2 ºC for 14 days, and the medium coloration was then observed.
2) Growth at 37ºC: This experiment was conducted to differentiate between the two species of Trichophyton based on their ability to grow at 37ºC, such as T. rubrum, which is unable to grow at this temperature, whereas T. mentagrophytes was able to grow under the same conditions [16].

2.7 Detection of Candida Species

Candida specimens examined initially by using wet mount preparation. Potassium hydroxide (10%) was applied to increase the visibility of fungal components by digesting proteinaceous material in the specimens [17]. The isolation of Candida spp. carried out on Sabouraud dextrose agar (SDA), which promote and facilitate Candida growth while suppress and inhibit the growth of many bacterial species due to its low pH. The plates were sealed with parafilm and then stored in the refrigerator for preservation. Isolates were subcultured onto CHROMagar Candida with the plate facing upright and incubated at 35 ± 2 °C for 72–96 hours. The results were recorded and documented. Yeast colonies were initially identified depending on their color, compared with standard color images provided by the manufacturer and reported by Mahmoudabadi et al [12].

2.8 Detection of Non-Candida Yeast

For isolation of Cryptococcus sp. and Rhodotorula sp., samples cultured on mycological media SDA as mucoid colonies at 35±2 °C underwent microscopic examination to observe cellular morphology. Briefly, a drop of LPCB was placed at the center of a clean glass slide. A small portion of the pure isolates of fungal colony was transferred using a sterile inoculating loop, gently mixed with the stain, and covered with a coverslip. The prepared slides were

examined under a light microscope to assess the morphological characteristics of the fungal cells, and the experiment was repeated using India Ink to observe encapsulated cells [1], [8].

2.9 Confirmatory Identification of Yeast Species using VITEK2 Technique

This technique was used to approve identification isolates with yeast colonies in SDA cultures which were identified microscopically as yeasts. The protocol was improved as following: Tests were performed from subcultures grown for 24 to 55 hrs on SDA plates. Inocula were adjusted to a no.2 McFarland standard. The VITEK ID-YST card consists of 64 wells with 47 fluorescent biochemical tests. They comprise 20 carbohydrate assimilation tests: The integrated VITEK 2 instrument automatically filled, sealed and transferred the cards into an incubator (incubation temperature was 35±2°C). Every 15 min the cards were automatically subjected to a fluorescence measurement. Each profile was interpreted according to a specific algorithm. Following a 15-hour incubation period, results were matched with the ID-YST database, leading to the final identification of the microorganism [18].

3 RESULTS AND DISCUSSION

3.1 Samples Examined During the Study

During the period from May 2025 to February 2026, 120 specimens, including onychomycosis swabs, hair fragments, nail clippings, and skin fragments, were collected from 100 patients with various types of dermatomycoses.

In total, the number of patients was 100 with 115 specimens. Clinical diagnosis of the patients was confirmed mycological (Fig. 1), results showed that dermatomycses were more common in females than males in 64 (55.65%) and 51 (47.83%) respectively With a significant difference at the probability level ($P < 0.001$), the data indicate that the distribution of fungal skin infection types differs significantly according to gender among the individuals under study, and this result was consistent with what was reached by Phan and Chandler [19]. Tinea manuum was the most prevalent, while tinea faciei was the least prevalent (Table 2) to have tinea manuum (23 cases; 20%) A distinctive, itchy, ring-shaped, scaly plaque appeared on it, accompanied by a burning sensation and healed center; tinea pedis (*12*cases; *10*.43 %) in which a condition of moist, flaky, and irritated skin appeared between the toes; tinea capitis (18 cases;15.65 %) presented with hair loss with scaling; tinea corporis (15 cases; 13.043%); presented with circular, scaly, red lesion with advanced borders on the neck, chest, and arm; tinea cruris (11 cases; 9.7%) presented with pruritus and red inflamed patches with sharp border in the groin; and tinea unguium or onychomycoses (8 case only; 6. 96%) presented with scaly and red areas in addition to thickened nails that are discolored yellow. Tinea manuum was the most prevalent kind of tinea. However, tinea capitis came in second, which contradicted the findings of Naseri et al [20], who found that the frequency of dermatophytosis was higher in females than males. However, Table 2 displays the number of samples based on the clinical kind of cutaneous mycosis.

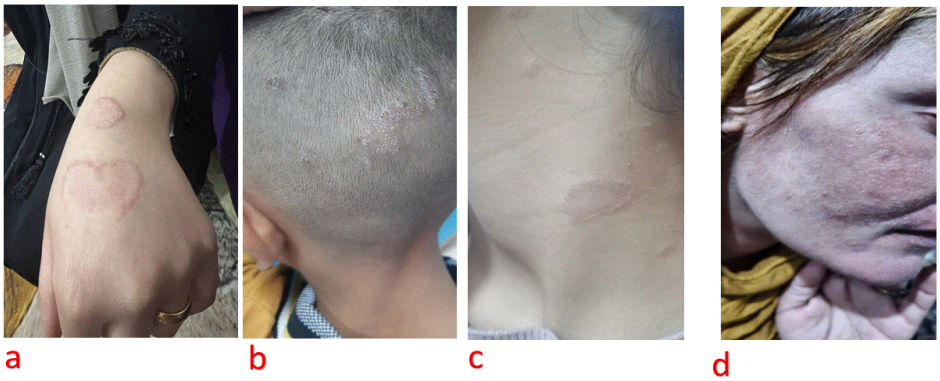

Figure 1: Clinical types of dermatophytosis: a) Tinea manuum showing red and scaly areas in an immunocompromised patient, b) Tinea capitis showing circular lesions with scaling, c) Tinea corporis showing circular erythematous scaly lesion

with advancing margin on the neck, d) Tinea faciei show an erythematous, scaly patch or annular plaque on the cheek and nose.

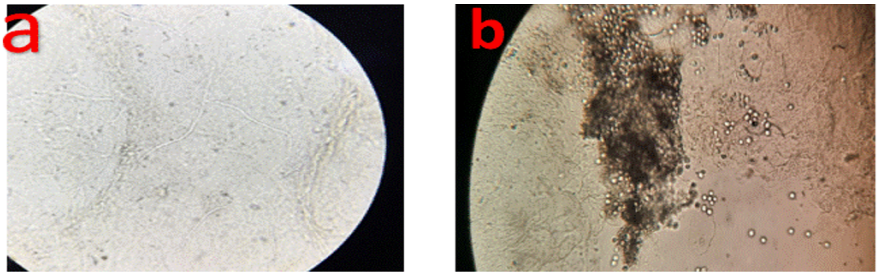

Figure 2: Direct Exam by KOH 10% shows: a) the hypha of a filamentous fungus from skin scrabs with tinea mannum, b) budding cell and destroyed skin tissue with tinea pedis.

Table 2: Prevalence of clinical features of dermatophytoses and onychomycosis in relation to the sex and age of patients.

Types of clinical infection	Sex		Sig.	Group of age (years old)		
	male	female		5-20	21-40	41-60
Tinea mannum	6	17	<0.05*	2	18	3
Tinea capitis	16	2		18	-	-
Tinea corporis	9	6		1	12	2
Tinea pedis	4	8		2	8	2
Tinea cruris	7	5		5	7	-
Tinea unguium	2	6		5	3	-
Tinea faciei	1	2		1	2	-
Tinea favosa	-	-		-	-	-
Onychomycosis	6	18		4	20	1
Total	51	64		115		

Note. *p < 0.05 $\chi^2 = 3.884$, df = 1

All specimens were examined directly with KOH 10% under light microscope to observe the presence of fungal hyphae and then cultured on plates with SDA medium to study the cultural characteristics. The results as a total showed that 115 (95.83%) were (+ve) for fungal isolates including 90 (78.26%) dermatophytes and 25 (21.74 %) opportunistic fungi. (Fig. 2).

Some negative results in direct microscopic examination and culture examination could be due to the following reasons: The small number of samples collected was insufficient to produce a positive result, or the samples were not taken precisely from the infected area or margins, but rather from the central part of the affected area, which may have had local immunity, resulting in the absence of dermatophytes [15]. Figures 2 and 3 depict a sample of immunocompromised people with tinea mannum and the hypha of a dermatophyte fungus from a skin swab. Non-prescribed antifungal drug usage by some individuals may affect the viability of dermatophytes but not their proliferation [14], [21].

3.2 Dermatophytes and Opportunistic Fungal Isolates

Findings appeared that the total number of fungal (positive) isolates were 115 (95.83%) included 90 (78.26%) dermatophytes when compared with reference standard culture results by assisted microscopy using Lactophenol alanine blue staining, 90 isolates of dermatophytes were identified by 4 species including Epidermophyton floccosum 32 isolates, Trichophyton rubrum 29 isolates, Microsporum canis 18 isolates and T. mentagrophytes 11 isolates in addition to opportunistic yeasts and molds represented by 25 isolates: Candida albicans 12 isolates, Cryptococcus neoformans 2 isolates, Rhodotorula mucilaginosa 4 isolates, Aspergillus nidulans 2 isolates, A. niger 2 isolates, Rhizopus arrhizus 1 isolate, Trichoderma longibrachiatum 1 isolate (found as etiological agent of tinea cruris) and Penicillium sp 1 isolate; and when using another two parameters; growth on PDA

medium and culture at temperature 37 ºC in SDA medium, The biochemical and physiological characteristics in Table 4 are used to help in the diagnosis of dermatophytes. T.rubrum demonstrated red pigments on PDA medium, whereas T. mentagrophytes and M. canis cannot. M. canis and T. mentagrophytes were capable of growing at 37ºC, while this feature it was absent in T. rubrum. T. mentagrophytes was capable of growth at 37 ºC, whereas T. rubrum cannot, which supports species differentiation [22]. All results were agreed to with Hasan [23].

The results presented that only 5 (4.17 %) specimens were negative, in culturing and biochemical tests. However, the appearance of negative results in culture may be due to the fact that some people with skin infection or tinea resort to using topical treatments randomly and without consulting a dermatologist, due to the discomfort caused by this infection, which may in rare cases lead to affecting the vitality of the skin fungi and their non-growth after culture [21]. However, Figures 3-6 illustrate the morphocultural characterization of some isolated dermatophytes and opportunistic fungal species.

All fungal species isolated from dermatomycoses are summarized by percentage in Table 3. However, Candida infections remain a major problem worldwide, especially among immunocompromised patients. Candida spp. are normal constituents of the microbiota that possess the ability to invade living tissues (opportunistic organisms) and cause cutaneous candidiasis [8]. Likewise, Aspergillus spp, which is generally harmless in its natural environment, and may become pathogenic immunocompromised patient [1], [24]. The results of this study are consistent with Hussain [24], who stated that people with weakened immune systems may be more prone to opportunistic infections, as well as to common infections that can affect healthy individuals. Fungal infections are generally opportunistic and primary. Opportunistic infections occur predominantly in individuals with weakened immune systems, whereas primary infections can develop in immunocompetent people [25]. However, several previous studies have indicated that opportunistic fungi cause skin infections [6], [19], [26]-[28].

In relation to dermatophytes and clinical types of dermatophytosis (Table 5), results showed that E. floccosum was the most prevalent species, with 32 isolates recorded, including the clinical type tinea mannum (which was the most prevalent type), followed by T. rubrum (29 isolates) which was more prevalent in tinea corporis, tinea pedis and tinea cruris.

These results were similar to those obtained by Ismael et al [6] in areas near the city of Al-Khalis. M. canis, however, came in third place and was prevalent in the clinical type of tinea capitis in which recent studies confirm this finding also [6], [19]. T. mentagrophytes appeared in 11 isolates with different clinical types, as these results were repeated in previous studies especially the latest study presented by the researchers Phan and Chandler [19].

Table 3: Percentage of dermatophytes and opportunistic isolates from patients with infected hair, nail and skin.

Fungus name	Percentage %	Total No.
Epidermophyton floccosum	27.82	32
Trichophyton rubrum	25.28	29
Microsporum canis	15.65	18
T. mentagrophytes	9.56	11
Candida albicans	10.44	12
Cryptococcus neoformans	1.74	2
Rhodotorula mucilaginosa	3.47	4
Aspergillus nidulans	1.74	2
Aspergillus niger	1.74	2
Rhizopus arrhizus	0.87	1
Trichoderma longibrachiatum	0.87	1
Penicillium sp.	0.87	1
Summation	100	115

Table 4: Differentiate among dermatophytes species according to some of physiological characteristics.

Species of dermatophyte	Growth potential on PDA medium	Growth at 37 ºC
Trichophyton mentagrophytes	-	+
T. rubrum	+	-
Microsporum canis	-	+

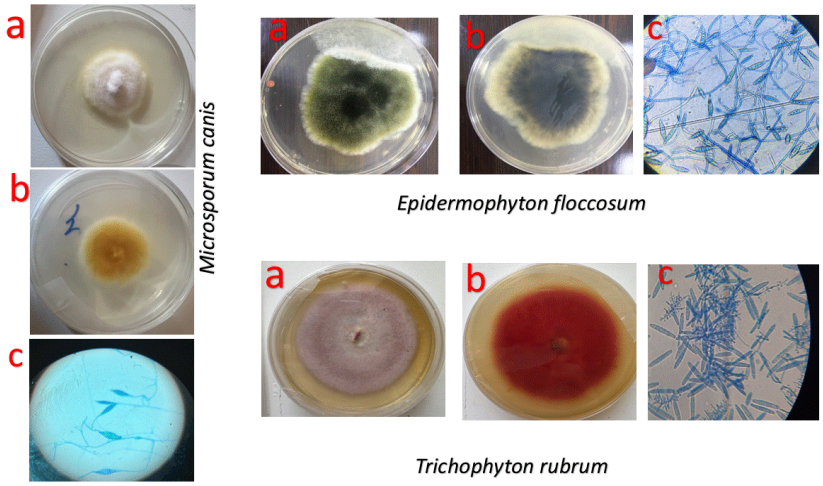

Figure 3: Dermatophytes species cultured on Sabouraud Dextrose Agar at 28±2 °C for 12 days: a) Top feature, b) Reverse, c) Microscopical feature with lactophenol cotton blue stain at 40 X.

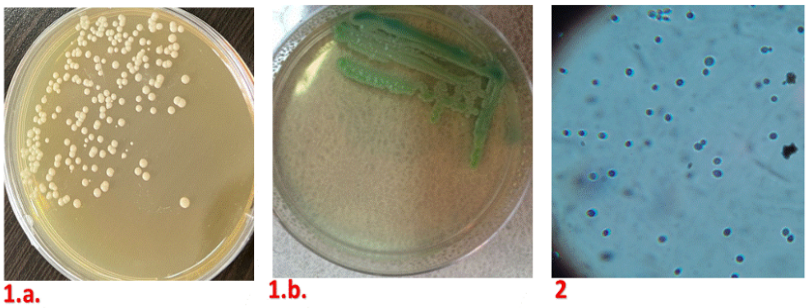

Figure 4: Morphological identification of *Candida albicans*: 1a) colony appearance on Sabouraud Dextrose Agar, 1b) characteristic green colonies on CHROM Candida Agar, and 2) microscopic observation showing budding cells stained with lactophenol cotton blue.

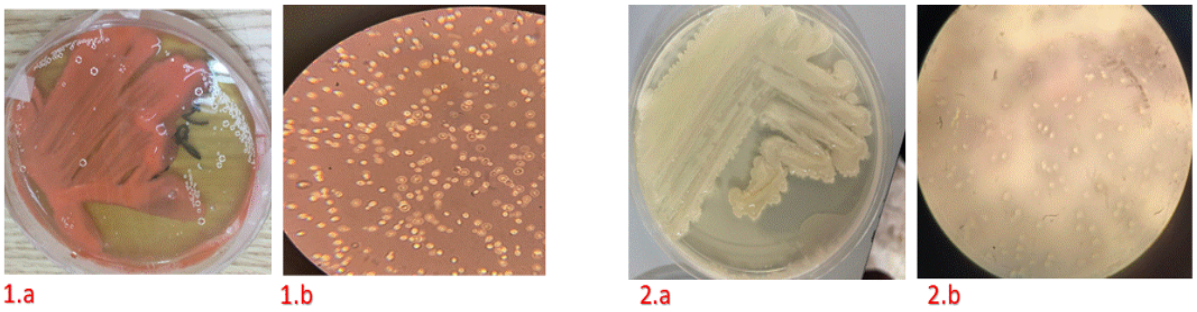

Figure 5: Morphological characteristics of yeast isolates: 1a) colony morphology of *Rhodotorula mucilaginosa* on Sabouraud Dextrose Agar, 1b) microscopic observation of its polysaccharide capsule by India ink staining, 2a) colony morphology of *Cryptococcus neoformans* on Sabouraud Dextrose Agar, and 2b) microscopic observation of its polysaccharide capsule by India ink stainin.

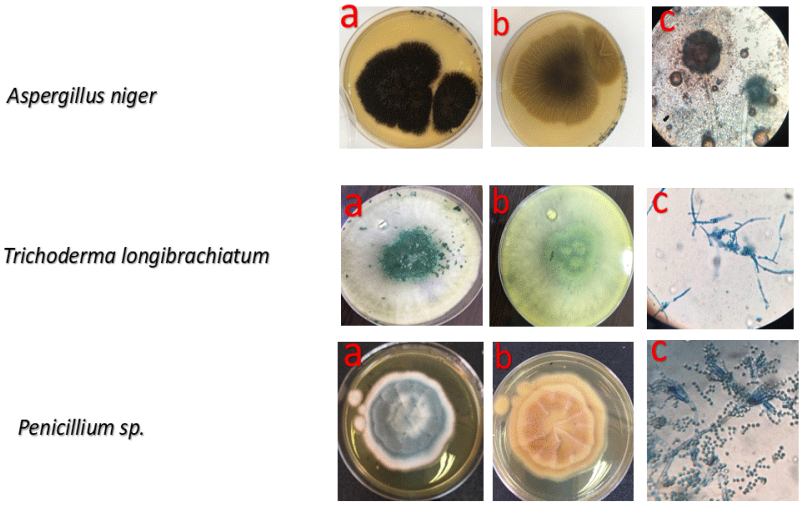

Figure 6: Opportunistic mold species cultured on Sabouraud Agar at 28±2℃ for 5-7 days: a) Top feature, b) Reverse, c) Microscopical feature with lactophenol cotton blue stain at 40 X.

Table 5: Dermatophytes species isolates in relation to clinical feature of cutaneous mycoses.

Types of clinical infection	E. floccosum	T. rubrum	M. canis	T. mentagrophytes	Trichoderma longibrachiatum	Total
Tinea mannum	21	1	0	1	0	23
Tinea capitis	0	0	15	3	0	18
Tinea corporis	2	10	1	2	0	15
Tinea pedis	2	9	1	0	0	12
Tinea cruris	0	8	0	3	1	12
Tinea unguium	7	0	0	1	0	8
Tinea faciei	0	1	1	1	0	3
Tinea favosa	0	0	0	0	0	0
Total	32	29	18	11	1	91

The results show that the distribution of dermatophytes among the clinical types was consistent with what was mentioned by DR Arora and Arora [1]. In addition to the 90 isolates of dermatophytes, there was another isolate of Trichoderma longibrachiatum isolated for the first time from a female patient with the tinea cruris, this species known to be a saprophytic that rarely causes opportunistic infections in skin [29]; previously, this species was isolated from the fluid puncture of an inguinal abscess and from a skin biopsy of an elderly male who was receiving a therapy of immunosuppressant types [30]. The reason this pathogen appears alongside other species of dermatophytes is likely due to its possession of virulence factors similar to those found in dermatophytes, enabling it to infect the skin [8]. Therefore, it is essential to conduct enzymatic and molecular studies of this species to determine its pathogenicity and identify suitable antifungal agents. These findings support global epidemiological data indicating a rise in infections caused by less common species of fungi, as well as changing patterns of antifungal resistance [19], [31].

Upon reviewing Table 1 in this study, we observe that some of the dermatophyte present were of animal or human source and this is confirmed by the city resident's interactions with animals, especially cats and dogs, even inside homes, which has increased the spread of infection through contact in addition to other health factors, including a weakened immune system due to pregnancy, diabetes, cancer, and the indiscriminate use of medications, among others [24], [32]. Therefore, decrease contact with animals is important to get high level of protection.

3 CONCLUSIONS

The present study confirms that dermatomycoses represent a diverse group of superficial fungal infections involving both dermatophytes and opportunistic fungal species. The combination of clinical evaluation, direct microscopic examination, and cultural identification was effective for the diagnosis and differentiation of fungal agents associated with cutaneous infections.

The predominance of Epidermophyton floccosum, Trichophyton rubrum, Microsporum canis, and T. mentagrophytes highlights the importance of species-level identification in understanding the epidemiology and transmission patterns of dermatophytosis. The detection of opportunistic yeasts and molds emphasizes their potential contribution to cutaneous infections, particularly in individuals with predisposing conditions.

The recovery of Trichoderma longibrachiatum from a case of tinea cruris represents an uncommon finding and suggests the need for further molecular investigations to confirm its pathogenic role and evaluate its antifungal susceptibility. Preventive strategies, including reduction of exposure to infected animals and improved awareness of transmission routes, may contribute to reducing the incidence of dermatophytic infections.

Overall, these findings support the importance of integrated diagnostic approaches and continuous epidemiological monitoring for the effective management and control of dermatomycoses.

ACKNOWLEDGEMENT

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ETHICS

We undertake to follow the ethical principles and institutional requirements guiding (Al-Khalis General Hospital and the private clinic) According to ethics book numbered (CEPEC/1378), this research and ensure the sign of personal declaration of responsibility prior to their specimen involvement in the research.

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