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Survey on common causative agents of dermatophytosis in Al-Bayda Libya

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Abstract: The aim of this study was to isolate and identify dermatomycosis causing dermatophysis in Al-Bayda, Libya. In this study 253 cases of skin infection were collected from the dermatology unit at EL- Thoura hospital in Al-Bayda, Libya. These cases clinically diagnosed as superficial mycosis ,130 (51.4%) were isolated from males and 123 (48.6%) were females. These cases were classified into 5 groups according to age (1-10, 11-20, 21-31, 31-40 and above 40 year). Our results showed that young ages were more liable to infection than adult. The cases involved in this investigation represented different occupations. The highest incidence was recorded between school children 40.3 %, followed by workers, farmers and others 29.2%, house wives 17.8% while it was 12.6% among children less than 5 years. Concerning the clinical and mycological finding, it was noticed that 74 (63.8%) cases gave positive culture while the remaining cases were negative. Scalp infection was the most common clinical finding, in this study Tinea capitis was the most frequent 42.8% cases, *Microsporum canis* and *Trich ophyton violaceum* were the most common fungi isolated. Tinea corporis and Tinea cruris were diagnosed clinically in 68 (26.8%) cases, 24.4% were from Tinea corporis while the fungus isolated were *Microsporum canis* 41% , *Epidermophyton floccosum* 14% and Tinea cruris with incidence of 0.8% in which *Trichophyton tonsurans* was the only fungus isolated . Onychomycosis and Tinea pedis were seen in 3.4% and the most important isolated agents were *Epidermophyton floccosum* 50%, followed by *Microsporum canis* and *Trichophyton rubrum* 25% for each. This study demonstrates that the prevalence of dermatophytoses in Al-Bayda cit, was high (47%), and was more common in males than females. The present study has also indicated that tinea capitis was the dominant (43%).

Keywords: Dermatophytosis, Tinea, Prevalence, *Microsporum* ,*Trichophyton* , *Epidermophyton*

INTRODUCION

In developing countries the prevalence of dermatophytosis has been reported by many authors, including (Carrillo-Munoz et al., 2008; Ghannoum et al., 2003). The disease is manifested as infection of keratinized tissues such as the epidermis of the skin, hair or nails, and is caused by dermatophytes. It can affect males and females regardless of age.The most important fungal genera implicated in superficial mycoses are Epidermophyton, *Microsporum*, and *Trichophyton* (Ameen, 2010; Popoola, Ojo, & Alabi, 2006). The excessive use of immunosuppressive drugs for controlling as well as non-infectious conditions allow these fungi to assume greater significance.

The infection by dermatophytes is facelifted by excretion of the enzyme keratinase that degrades keratin in superficial skin tissues. Thus, their infections are generally cutaneous and restricted to the non-living layers of the skin. Dermatophytes lack the ability to invade deeper tissues or organs of the host. In chronic conditions, however, these fungi may invade deeper tissues, particularly in concurrent infections

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with other organisms. Because of their ring-like appearance, typical infections by dermatophytes are generally called ringworm infections, or sometimes called 'tinea infections' which are named according to the location of the lesions on the body (e.g. tinea capitis, tinea corporis, Tinea pedis, Tinea cruris, Tinea unguium, Tinea manuum, and Tinea barbae) . Tinea infections are ubiquitous, but they are more common in tropical regions and may reach epidemic proportions in places with higher humidity, over-population, and poor hygienic living conditions (Popoola et al., 2006; Weitzman & Summerbell, 1995).

In Libya, the hot and humid climate makes dermatophytosis a very common cause of superficial fungal infections of the skin. (Malhotra, Garg, Kanwar, & Nagrajan, 1979) However, it is difficult to reliably ascertain the overall incidence and prevalence of these infections, as studies of one region of the country may not represent the overall disease pattern. Furthermore, incidence and prevalence figures may only be representative of the population sampled, which may have associated risk factors for infection. Identification of the causative agents may give valuable clues to the risk factors and epidemiological trends, in Libya few studies were conducted on the aetiology of superficial fungal infections. Tinea capitis (ringworm of the scalp) is the most common fungal infection in children, more than 90% of the infections are caused by *Trichophyton tonsurans*, and less than 5% are caused by *Microsporum* species (Andrews & Burns, 2008). Tinea corporis infections usually appear on the trunk, limbs, and occasionally the face in the form of annular, scaly patches or plaque with raised, scaling border and central clearing (Weitzman & Summerbell, 1995).T. rubrum is the most common cause worldwide (Andrews & Burns, 2008).Tinea corporis is more commonly caused by *T. tonsurans* and other causative dermatophytes including *M. audouinii, T. mentagrophytes, T. verrucosum,* and *E. floccosum* (Denk, 2007). Tinea cruris infects the groin, perianal, and perineal areas particularly in adolescent, young adult men, and in post-pubertal females, and *T. rubrum* (Chakrabarti, Sharma, & Chander, 1992) is the most common causative agent followed by *E. floccosum* .Tinea manuum appears as diffuse dry scaling lesions with accentuation of the flexural creases of the palms of the hands, and *T. rubrum* is the commonest infecting agent (Degreef, 2008).Tinea pedis usually appears in the interdigital clefts, sometimes spreading to the soles, and is due to three different fungal species, *T. rubrum T. mentagrophytes* and *E. floccosum* Tinea unguium (Onychomycosis) is a fungal infection of the nail caused mainly by *T. rubrum* and *T. mentagrophytesvar interdigitale* (Svejgaard & Nilsson, 2004).

MATERIALS AND METHODS

Sample Collection: 253 clinical samples were collected from patients visiting the Dermatology Department of EL- thoura hospital in Al-Bayda, Libya, 130 of which were isolated from males and 123 from females. Before collecting the sample, the infected area was cleaned with 70% ethanol (Martin & Kobayashi, 1993). The skin and fingernail samples were obtained by scraping the lesion with a sterile blade, and broken hairs were obtained from the margin of scalp lesion with forceps and transferred to sterile folded papers. The papers were appropriately labeled with the age, sex, date of collection, code of a patient, and location of infection and were examined in the Microbiology Laboratory of the Dermatology Department of EL- thoura hospital in Al-Bayda.

Direct microscopical examination: A portion of each sample was mounted in a drop of an aqueous solution of 10 -20 %(w/v) potassium hydroxide (KOH) on a clean microscopic slide. After 5 minutes of mounting, the preparation was examined under low (×10) and high (×40) power magnification for the presence of fungal elements (e.g. arthrospores, septate hyphae, spores) (Fig .1)

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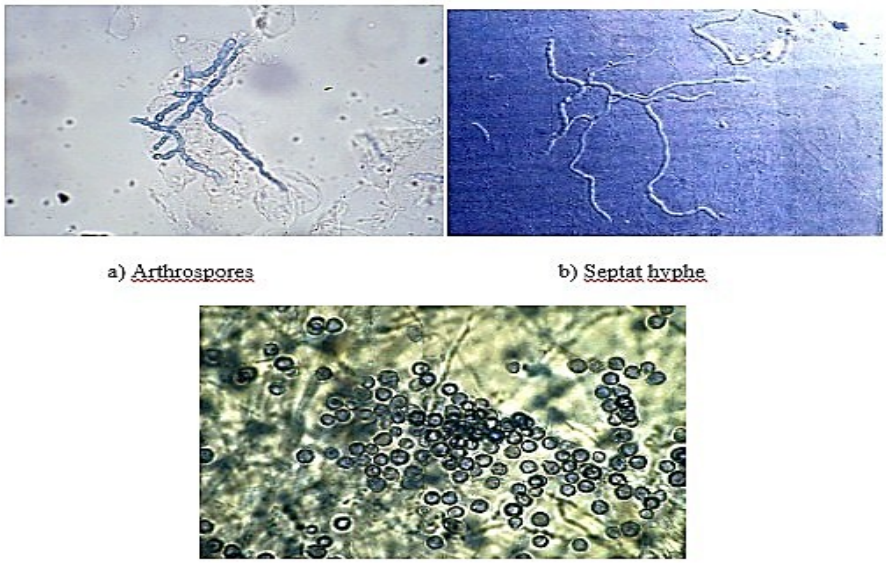

Figure (1). (a , b ,c) : examined under low (×10) and high (×40) power magnification for the presence of fungal elements (a) Arthrospores (b) Septat hyphe (c) Spores

Isolation of causative fungi : The remaining portion of each clinical sample was cultured irrespective of the negative or positive direct microscopic examination results onto plates of Sabouraud's dextrose agar containing chloramphenicol with and without cycloheximide (Oxoid, Basingstoke, England) which were prepared according to the manufacture's instruction.(GEO, 1999). All inoculated plates were then incubated at an inverted position for 2–4 weeks at 25–30°C aerobically. Culture plates were examined twice a week for any fungal growth. (Gameel & ALsenosy, 1996). Colonies suspected of dermatophytes were sub-cultured onto Sabouraud's dextrose agar of chloramphenicol cycloheximide for the production of spores.

Identification of the isolated dermatophytes
Cultures of dermatophytes were identified by examining macroscopic and microscopic characteristics of their colonies. For this purpose, texture, rate of growth, topography, and pigmentation of the front and the reverse side of the culture were employed for the macroscopic identification, front and the reverse side of the culture were used for the macroscopic identification. Microscopic identification of mold isolates was performed by placing pieces of a colony from SDA and/or DMT to clean microscopic slides and stained with blue lactophenol cotton. After placing a cover slip, each preparation was observed microscopically to distinguish between dermatophytes causing disease (*Microsporum*, *Trichophyton*, *Epidermophyton*) (Fig. 2).

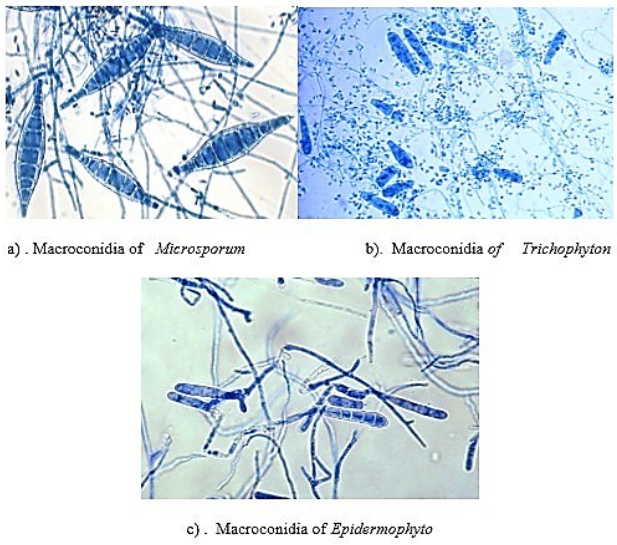

Figure (2). (a, b, c): Microscopical examination of cultures to distinguish between dermatophytes causing disease, (a) *Microsporum,(b) Trichophyton, (c) Epidermophyton*.

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RESULTS

In the present study, a total of 253 clinical samples were collected from suspected cases of dermatophytosis from the dermatology unit at EL-Thoura hospital in Al-Bayda, Libya, of which 130 (51.4%) were males and 123 (48.6%) were females. The ages of study subjects ranged from 1 year to above 41 years.
The details regarding clinical manifestation and sex of study subjects are given in Table 1, which explains the relationship between age, sex, and incidence of the disease.
The prevalence of dermatophyte infection has variable frequencies with respect to the age and gender grouping of participants. The highest prevalence was seen in age group 1–10 years, with a rate of injury of 34.4%. The least was in age group 11-20 years (24.1%) and was more in females than males.

Table (1). Shows the relationship between the Age, Sex, and rate of injury dermatophytosis.

Age	Sex				The total number	Percentage%
	Females		Males			
	%	Number	%	Number		
1-10 Years	24.4%	30	43.8 %	57	87	34.4 %
11-20 Years	26 %	32	22.3%	29	61	24.1%
21-30 Years	22.1%	27	16.2%	21	48	18.9%
31-40 Years	15.4%	19	7.7 %	10	29	11.5%
above 41 years	12.2%	15	10 %	13	28	11.1%
The total	100%	123	100%	130	253	100%

Table: (2) shows the relationship between different occupations and the incidence of the disease. The highest incidence was recorded between school children which were 102 cases (40.3 %) followed by workers, farmers, and others (74 cases) (29.2%), then housewives 45 cases (17.8%).

Table (2). Shows the relationship between different occupations and incidence of the disease.

The total cases	different occupations	Housewives	School students	Children	occupations
253	74	45	102	32	Number
100%	29.2%	17.8 %	40.3%	12.6%	Percentage%

From the 253 clinical samples that were collected, 197 (77.9%) had fungal elements as seen under microscopic examination (KOH +), 56 (22.1%) were (KOH -), while 119 (47%) clinical samples were culture positive.
Several species of dermatophytes were isolated in the present study. The predominant pathogens were *M. canis* (35.3%), which was responsible for 22 cases of tinea capitis, 13 cases of tinea corporis, 5 cases of tinea manuum, and 1 for each of tinea barbae and tinea pedis. *M. canis* was followed by *T. violaceum* (21%), which was responsible for 20 cases of tinea capitis, 3 cases of tinea corporis, and 1 for each of tinea barbae and tinea unguium. *T. rubrum* (20 2%) was isolated from 16 cases of tinea unguium.

T. verrucosum was isolated from 8.4% cases of tinea capitis, 2 cases of tinea corporis and tinea unguium, and 1 case of tinea manuum.
E. floccosum (6.7%) was isolated from 4 cases of tinea corporis, 2 cases of tinea pedis, and 1 case for each of tinea unguium and tinea manuum.

T. mentagrophytes (3.4%) was responsible for 2 cases of tinea corporis, 11 cases for each of tinea unguium and tinea barbae, whereas *T. schoenleinii* and *T. tonsurans* were the least common (2.5%) for both. (see table 3, and fig. 3,4).

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Table (3). Shows the types of dermatophytes isolated and their rates of spread in the present study.

NO	Types of dermatophytes isolated	Number	Percentage%
1	*Microsporum . canis*	42	35.3 %
2	*Trichophyton. violaceum*	25	21 %
3	*Trichophyton. rubrum*	24	20.2%
4	*Trichophyton. verrucosum*	10	8.4 %
5	*Epidermophyton. floccosum*	8	6.7 %
6	*Trichophyton. mentagrorphytes*	4	3.4 %
7	*Trichophyton. schoenleinil*	3	2.5 %
8	*Trichophton. tonsurans*	3	2.5 %
The total		119	100 %

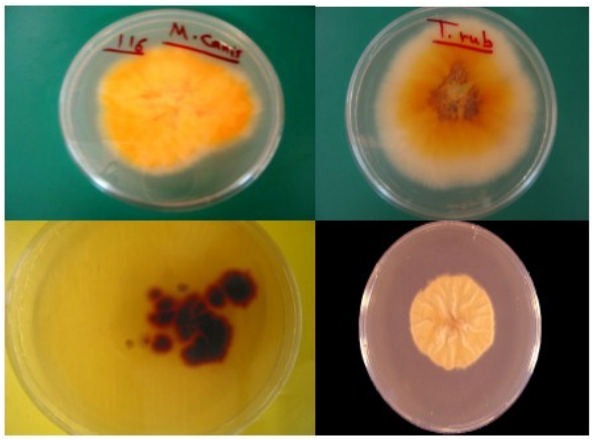

Figure (3). Species of dermatophytes were isolated in the present study of the predominant pathogens, *M. canis*, *Trichophyton. rbrum*, *T. violaceum, T. verrucosum*.

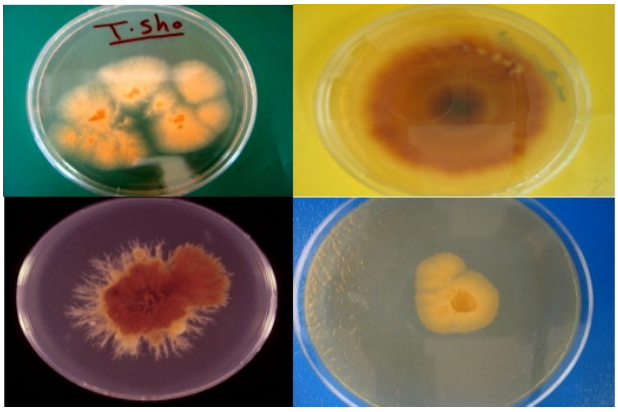

Figure (4). Species of isolated dermatophytes which were the least common, *T. mentagrorphytes Epi. floccosum*, *T. tonsurans*, *T. schoenleinil*.

DISCUSSION

Dermatophytosis infections are more prevalent in the developing world. The present study attempted to determine the dermatophytosis infections and identify dermatophytes causing disease in (Al-Bayda) Libya. Of the 253 clinical samples collected from the dermatology unite at EL- Thoura hospital in Al-Bayda, Libya, 130 (51.4%) of the samples were isolated from males and 123 (48.6%) from females.

The present study showed that the age group 1-10 years was more sensitive to dermatophytes infections than other age groups. Infection was more common in males (43.8%), while in females it was 24.4%. Similar results were reported by earlier researches (Ellabib & Khalifa, 2001; Falahati, Akhlaghi, Lari, & Alaghehbandan, 2003; Simpanya, 1989). This could be due to poor awareness about hygiene and increased susceptibility to the dermatophyte at a younger age, or because of lower sebaceous gland secretion which may have an inhibitory effect on dermatophyte infection in older age. Dermatophytes were detected in 197 (77.9%) samples by KOH wet mount, and 119 (47%) samples were culture positive for dermatophytes. This is similar to what was reported by (Svejgaard & Nilsson, 2004 ; Fralco *et al*., 1991).

Of the total number of 119 dermatophytes isolates in the present study, *M. canis* was the predominant dermatophyte (35.3%) identified, followed by *T. violaceum* (21%), *T. rubrum* (20 .2%), *T. verrucosum* (8.4%), and *T. mentagrophytes* (3.4%). Similar results were reported by earlier researches . However, this finding is contrary to the observations by others in which a reverse trend has been reported (Al-Sogair, Al-Humaidan, & Moawad, 1989; Falahati et al., 2003; Shtayeh & Arda, 1985).

T. schoenleinii and *T. tonsurans* were in-

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volved only in 2.5% of the cases. This is similar to the finding by (Ellabib & Khalifa, 2001). Among *Trichophyton* species, *T. violaceum* accounted for 21% of the total isolates, and our finding was compatible with studies carried out in several other African countries (Gargoom, Elyazachi, Al‐Ani, & Duweb, 2000; Lange, Nowicki, Barańska‐Rybak, & Bykowska, 2004), and several Asian countries (Ali‐Shtayeh, Salameh, Abu‐Ghdeib, Jamous, & Khraim, 2002; Hussain, Aman, Haroon, Jahangir, & Nagi, 1994).

T. violaceum has been reported as one of the endemic dermatophytes in the horn of Africa and Asia (Ameen, 2010). Although we have no explanation for the small number of cases of *T. schoenleinii* and *T. tonsurans* as dominant dermatophytes in the present study as opposed to previous studies in East Africa, the heterogeneity in the distribution of dermatophytosis, their etiologic agents, and the predominating clinical manifestation patterns in different parts of the world have been attributed to factors of geographic location, climate, overcrowding, health care, immigration, environmental hygiene culture, and socioeconomic conditions as has been postulated by (Havlickova, Czaika, & Friedrich, 2008).

Ep floccosum (6.7%) was present in more cases of tinea pedis, this was similar to the findings of (Ellabib & Khalifa, 2001). Species identification of tinea capitis showed that *M. canis* (22 cases; 43%) was the most common dermatophyte, followed by *T. violaceum* (20 cases; 39.2%), *T. verrucosum* (5 cases; 10%), *T. rubrum* (2 cases; 4%), then *T. schoenleinii* (2 cases; 4%).

These results converged with (Zaini & Ghagari, 1989) who reported 45.6% for tinea capitis, and with (Gargoom et al., 2000) who reported 45% for tinea capitis, but are higher than the results of (Amer, Taha, Vet, Zeinab Tosson, & El‐Carf, 1981) who reported 36%, and (Shtayeh & Arda, 1985) who reported 38.7 % .Tinea corporis was the second most frequent infection in the study region with an incidence of 24.4%. The infection is mainly caused by *M. canis*, followed by *Ep. floccosum*, *T. violaceum*, and *T. rubrum*. Similar results were reported by (Ellabib & Khalifa, 2001; Karmakar, Kalla, Joshi, & Karmakar, 1995), but differed from (Amer et al., 1981).

Tinea unguium was the third most common infection (16.8%). Tinea unguium might result from wearing socks and shoes for a long period providing damp conditions, especially in inter-digital spaces. The infection is mainly caused by *T. rubrum* (80% of the cases), followed by *T. verrucosum* (10%), *Ep. floccosum* (5%), and *T. violaceum* (5%) which was more common in females than males. Similar results were reported by (Khafagy, Taha, & El-Gothamy, 1998) who reported that tinea unguium (15.6 %) was mainly caused by *T. rubrum*. These results differed with (Shibaki & Shibaki, 2003) who found that tinea unguium was 20.7 %.

The study indicated a low tinea cruris (0.8 %), only caused by *T. tonsurans*. This result was different from (Singh & Beena, 2003) who reported that the infection rate of tinea cruris was 12%, caused by *T. rubrum*. (Omar, 2004) found that tinea cruris was 83.9%, mainly caused by *T. rubrum* and *Ep. floccosum*.

In the present study, as opposed to previous studies, the heterogeneity in the distribution of dermatophytosis, their etiologic agents, and the predominating clinical manifestation patterns in different parts of the world have been attributed to factors of geographic location, climate, overcrowding, health care, immigration, environmental hygiene culture, and socioeconomic conditions . These variations confirm that the etiologic agents may differ in relation to dermatophyte host prefer-

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ence, occupation, and cultural habits with respect to gender and age of the studied population. Living with domestic animals or farming exposes individuals to zoophilic dermatophytes.

CONCLUSION

This study demonstrates that the dermatophytes isolated belonged to 8 species. *M. canis* (35.3%) was the most common pathogen in the study samples, whereas *T. schoenleinii* and *T. tonsurans* were the least common (2.5%).

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دراسة انتشار الفطريات الجلدية في مدينة البيضاء بمنطقة الجبل الاخضر

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المستخلص: الهدف من هذه الدراسة هو عزل وتحديد مرض التهاب الجلد في البيضاء، ليبيا. في هذه الدراسة، تم جمع 253 حالة من حالات العدوى الجلدية من وحدة الأمراض الجلدية في مستشفى الثورة في البيضاء، ليبيا. شخصت هذه الحالات سريريًا على أنها فطريات سطحية، وتم عزل 130 (51.4 ٪) من الذكور و123 (48.6 ٪) من الإناث وصنفت هذه الحالات إلى 5 مجموعات حسب العمر (1–10، 11–20، 21–31، 31–40 وما فوق 40 عامًا). أظهرت نتائجنا أن الأعمار الصغيرة كانت أكثر عرضة للإصابة من البالغين. تمثل الحالات التي فحصت مهنا مختلفة. وسجلت أعلى نسبة بين فئة أطفال المدارس 40.3 ٪، تليها فئة العمال والمزارعين وغيرهم 29.2 ٪، وربات المنازل 17.8 ٪ بينما كان 12.6 ٪ بين الأطفال أقل من 5 سنوات، بخصوص الكشف السريري والفطري، لوحظ أن 74 حالة (63.8 ٪) أعطت عزلة إيجابية في حين أن الحالات المتبقية كانت سلبية. كانت عدوى فروة الرأس Scalp infection هي الأكثر شيوعًا في هذه الدراسة كان التهاب Tinea capitis أكثر الحالات انتشارًا بنسبة 42.8٪، وكان كل من *Microsporum canis* و *Trichophyton violaceum* أكثر الفطريات شيوعًا. تم تشخيص Tinea corporis و Tinea crories سريريًا في 68 (26.8٪) حالات، 24.4٪ كانوا من Tinea corporis بينما الفطريات المعزولة كانت 41 *Microsporum canis*٪، 14 ٪ *Epidermophyto floccosum* وسعفة بنسبة 0.8٪ حيث كان *Trichophyton tonurans* هو الفطريات الوحيدة المعزولة. شوهدت الفطريات Tinea pedis في 3.4٪ وأهم الفطريات المعزولة هي *Epidermophyton floccosum* ٪ 50، تليها *Microsporum canis* و *Trichophyton Z rubrum* 25٪ لكل منهما. توضح هذه الدراسة أن معدل انتشار التهاب الجلد في البيضاء كان مرتفعًا (47٪) وكان أكثر شيوعًا عند الذكور منها عند الإناث، أشارت الدراسة الحالية أيضًا إلى أن tinea capitis كان هو الغالب (43٪).

الكلمات المفتاحية: انتشار، الفطريات الجلدية، مرض التينيا، تينيا الرأس، فطر *Microsporum* ، فطر *Trichophyton*، فطر *Epidermophyton*.

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