e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(9); 278-285

Original Research Article

Elucidation of Dermatophyte from a Tertiary Care Teaching Hospital through Currently Available Methods

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Received: 25-06-2023 / Revised: 28-07-2023 / Accepted: 30-08-2023

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Conflict of interest: Nil

Abstract:

Aim: To detect the typical dermatophyte that causes a fungal infection through traditional microscopic (KOH) and cultural techniques.

Methods: Patients with T. corporis, T. cruris, T. ungium, T. pedis, and T. barbae, aged 6 to 70 years, were included in this prospective study, which was conducted over a year. Microscopic analysis of skin and nail samples taken from the patient's body revealed the presence of dermatophytes. To observe under a microscope, cotton blue and KOH concentrations of 10–30% were both utilized. For statistical analysis, the SPPS version 20 was used to analyze the elucidated data.

Result: A total of 156 patients, who visited the Department of Skin and VD, KIMS Hospital, Bhubaneswar, were enrolled in the study. It was observed that a fraction of 59% of individuals were positive through direct the application of KOH, whereas a fraction of 52% of individuals were positive through cultural method. Interestingly few of those direct positives did not belong to the positive patients during the culture method. A maximum of T. mentagrophytes with a frequency percentage of 28% were observed during the study.

Conclusion: Trichophyton rubrum infections are prevalent in patients with tinea corporis. Then T.violaceum, T.tonsurans, and T.mentagrophytes infection occurs. Patients between the ages of 21-30 had the highest infection rate. The severity of elderly people between the ages of 61 to 70 years increases if the diagnosis and treatment are delayed.

Keywords: Dermatophytes, KOH, Culture, Diagnosis.

Introduction

Dermatophytes are the most common infection in humans, and most scalp, skin, and nails are infected with these dermatophytes. These dermatophytes are closely related to fungi that can invade the keratinized tissues of the above [1]. It includes three genera, i.e., Epidermophyton, Microsporum, and Trichophyton. These fungi colonize in the keratin tissues and are repeatedly restricted to the nonliving cornified layer of the upper cell layer of the tissues. Dermatophytes are also associated with secondary bacterial infections, leading to systemic skin infections. According to WHO, the prevalence rate of superficial mycotic infection worldwide has been found to be 20-25%. In South India, dermatophyte infections are commonly encountered in more than 50% of patients attending dermatology outpatient departments [2]. Tinea infections are prevalent globally, but they are common in the tropics and in geographical areas with higher humidity, overpopulation, and poor hygienic living conditions. It is transmitted by either direct contact or indirect contact (fomites). An increasing frequency of dermatophytosis has been observed during the last two decades, especially in immunocompromised patients such as AIDS, diabetes mellitus, cancer and organ transplantation patients, etc. Dermatophytosis generally responds well to topical antifungal therapy, although systemic therapy would be required for extensive infections or infections affecting the nails or scalp. The present study was conducted to isolate and identify dermatophytes from skin, hair, and nail samples of clinically suspected cases of dermatophytosis by KOH and culture on SDA and PDA media.

Materials and Methods

This study was carried out on 156 clinically suspected cases of Dermatophytosis in the age group of both sexes, attending the outpatient department of Skin & VD, KIMS Hospital, Bhubaneswar, Odisha, from March 2021 to April 2022. Inclusion criteria: Clinically suspected cases of dermatophytosis of all

age groups attending dermatology OPD were included in the study. However, cases that are not suspected to be dermatophytosis were not included in the study. All the details like age, sex, occupation, duration of presenting complaints, and site of the suspected lesion were taken in a pre-structured proforma.

All the clinical samples were collected from the skin and VD department, KIMS Hospital, Bhubaneswar. Two types of samples were taken for this study: skin scraping and nail clipping collected with a sterile swap stick and blunt scalpel for scraping the skin or nails of affected patients. For preliminary diagnosis, the microscopic examination was carried out in the Department of Microbiology, KIMS Hospital, Bhubaneswar. Dermatophytes were identified by translucent, non-pigmented, septate mycelium and arthrospores. Then, the remaining samples were cultured in Sabouraud Dextrose Agar medium (SDA) and whenever the growth was observed in the culture tubes, it was cultured by Potato Dextrose Agar (PDA). Sterilized equipment was used to avoid contamination by non-pathogenic fungi and bacteria.

The equipment and the media were used for the collection of specimens from the patients and the isolation and identification of the etiologic agents. Materials like; Ultraviolet Lamp, Wood Lamp, Epilating forceps, Nail Clippers, Scalpel, Scissors, inoculating needle, sterile test tubes and Petri-dishes, clean slides, sterile cottons were used for collection and culture the samples.

Culture Media

For the isolation and identification of the etiologic agents Sabouraud Dextrose Agar and Potato Dextrose Agar media were used. Both these culture medium for the growth of fungi had a high concentrated (20 gm per litre, Himedia, Mumbai.) of either glucose or dextrose and also contains mycological peptone.

The medium has a low pH 5.0, which inhibits the growth of most bacteria. Antibacterial agents Gentamicin (50mg per liter of medium) were added to augment the antibacterial effect.

Process of materials for culture

Clinical materials were collected from patients suffering from various types of dermatomycoses. Proper collection of clinical materials was very important for both direct examination and culture. The following types of specimens were included.

Skins scrapings: Scrapings were obtained from the lesions involving the skin with a sterile scalpel after carefully washing the site with 70 percent alcohol. Scrapings taken from active border areas of lesions were placed in a sterile petri-dish for laboratory examination and culture. In some lesions vesicles were present, which were carefully clipped off with small sterile scissors and successful microscopic and cultural examination was made.

Nail clippings: Ringworm-infected nails were found to be thickened and deformed. Clippings of nails, especially near the bed of the nail were collected in a sterile Petri-dish for microscopic examination and culture. The patients suffering from tinea capitis and tinea favosa were examined under normal light for areas without hair, scaling, crust formation, hair stumps and erythema. The tinea capitis patients were then subjected to Wood's light examination in a dark room to determine the fluorescence. The basal portion of the hair or the hair tufts were collected as the fungus is usually found in this area.

KOH applied microscopic examination of clinical materials:

The clinical material so obtained was dissolved in two drops of 10 percent potassium hydroxide on a glass slide. If the nail clippings were thick 20 percent potassium hydroxide was used. After putting the cover slip the slide was heated for few seconds over spirit lamp taking care that the material should not boil. The preparation was observed under the low power of the microscope in reduced light. Presence of mycelia fragments and the distribution of spores inside the hair were noted. For detailed microscopic morphology of the etiologic agent in clinical material, the slide preparation was studied under the high power of the microscope and the observations were recorded (Fig 2).

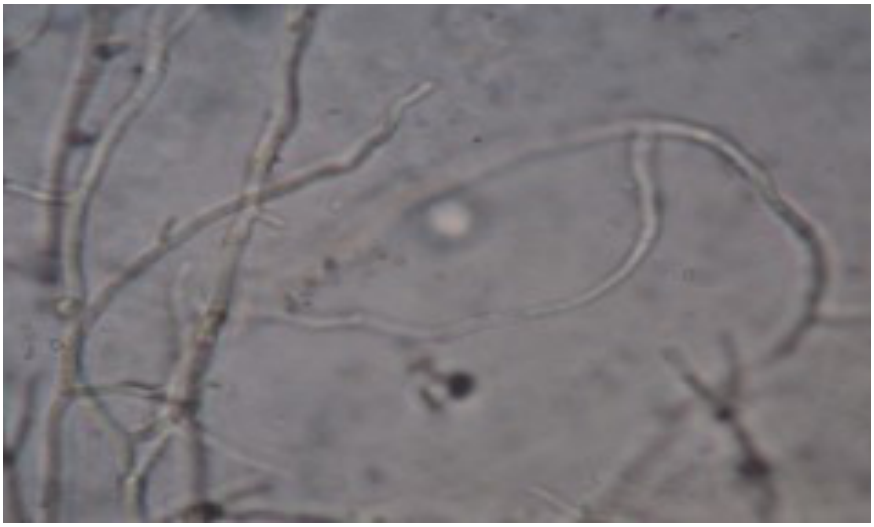

Figure 1: Direct identification of dermatophytes using KOH solution; branched hyphaes were observed in the KOH application

Culture of the Clinical Specimens:

Sabourauds Dextrose Agar (SDA) with Gentamicin medium was used for the isolation of the dermatophytes from the clinical material. The agar slants were inoculated by placing skin scrapings or nail clippings with the help of a sterile needle on the slant surface of the medium.

All the tubes inoculated were incubated at room temperature at 30 ℃ for three to four days. Slants were examined every four to six days (Fig). If any saprophytic fungus appeared, the suspected colony of dermatophyte was transferred to other slants. Inoculate the specimen as soon as possible after it is received in the laboratory. Place the specimen onto the center of the agar with sterile forceps. Press carefully to ensure firm contact with the agar surface. Replace the cap but do not tighten it completely.

Incubate the inoculated media at 30℃ for 4 days. (Do not incubate cultures at 37℃) Examine the culture daily for a change in the color of the medium and evidence of fungal growth. Expected cultural response on Dermatophyte Test Medium at 30℃ after 2-7 days. Escherichia coli Inhibition, partial to complete are mycelial fungi that possess keratolytic properties that allow them to invade skin, nails, and hair. The dermatophytes test Medium incorporates antibiotics that suppress the growth of saprophytic fungi and contaminate bacteria while allowing the growth of dermatophytic fungi. Dermatophytes are presumptively identified by gross colonial morphology and the production of alkaline metabolites which cause a color change in the medium from yellow to red.

Identification of the Growth of the Dermatophytes (Microscopic After Culture):

The skin sample was applied for KOH mounting and observed in the low to high magnificent microscope i.e., in 10X, 40X and 100X. By the application of KOH, the fungal elements seen were only the fungal hyphaes. A little of the sample was taken for cultural growth by means of an SDA medium. The sample along with the SDA media, was kept in the BOD at 30 ℃ for 3 to 4 days. After that, the colony morphology was observed (Table 2). The colony morphology was seen as: White fluffy growth, velvety red pigment on reverse, which is known to be Trichophyton species, white fluffy with reddish in the middle, yellow pigmentation was also seen sometimes when the colony morphology was seen as black in color, it was known to be contaminated otherwise the above all colonial morphology showed the +ve growth.

The fungus which was identified as positive was taken for microscopic examination (Fig 2). For microscopic examination, take the well-grown tube, sterile scalpel, Lacto phenol cotton blue, clean slide and cover slip. Applied 2 to 3 drops of lactophenol cotton blue on the clean slide and taken little amount of fungus from the cultured tube. With the help of a botanical needle, the material was spread over the slide so that clear examination could be possible. After teasing the whole materials, covered it with the cover slip and was spread over the slide so that a clear examination could possible.

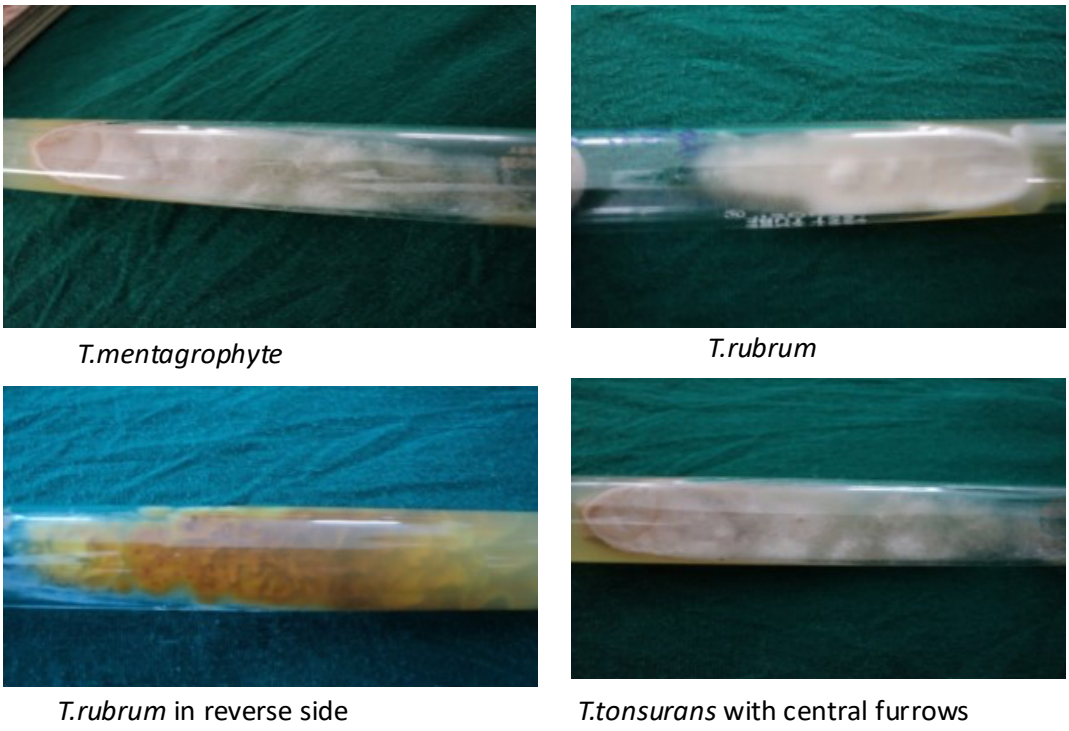

Figure 2: Identified cultured tubes by SDA & PDA method showing T. mentagrophyte, T. rubrum and T. tonsurans

It was observed that in two cases, the Trichophyton agent was the common one because in skin as well as in nail samples after growth and microscopic examination Trichophyton rubrum and T.mentagrophytes were observed in the microscope.

In T.rubrum there were pencil shaped macroconidia observed. T.mentagrophytes was also seen i.e. like clusters of macroconidia. Septate spindle shaped macroconidia were observed. An interesting thing seen to be confirmed was T.mentagrophytes were Spiral hyphae which were seen with macroconidias.

When it was confirmed to be Microsporum canis and Microsporum audinii. Epidermophyton species were also seen i.e yellowish green powdery in cultural morphology and club shaped macroconidia in microscopic examination. The microscopic and colony morphology of dermatophytes were described (Table 1).

Table 1: Microscopic and colony morphology of the dermatophytes

Sl. No	Dermatophytes	Colony morphology	Microscopic morphology
1.	T. rubrum	Creamy cottony pigment on reverse	Tear shaped microconidia observed
2.	T. interdigitale	White powdery surface and yellow to brown in the reverse tube	Grape-like clusters observed
3.	T.tonsurans	Central furrows were observed	Thick-walled, irregular macroconidia
4.	T.mentagrophytes	Variable pigments observed	Clusters of microconidia with cigar shaped macroconidia and tails
5.	T.schoenleinii	Smooth, waxy and brownish	Hyphal swelling, chlamydospores and favic chandelier
6.	T.violaceum	Slow growing, waxy, violet to purple pigment	Distorted hyphae and conidia rare
7.	Mi. audouinii	Velvety, brownish slow growing	Thick walled chlamydospores, conidia rare and irregular
8.	M.canis	Cottony, orange pigment on reverse were seen	thick-walled spindle-shaped macroconidia 15 septa
9.	M.gypseum	Powdery, buffy coloured	Thin- walled macro conidia with 4 to 6 septa were observed
10.	E.floccosum	Yellowish-green, powdery observed	Club-shaped macroconidia in clusters were observed

Note: T: Trichophyton, M: Microsporum, E: Epidermophyton

Results

A fraction of 46% was from the age group of 21-30 years out of the total 156 suspected cases of dermatophytes. Other age groups and the frequency of distributions were described (Table 2)

Table 2: Age- and gender-wise distribution of cases

Age group	Sex distribution (%)		Total (%)
	Male	Female	
10-20	15	06	21 (13.46)
21-30	35	09	44 (13.46)
31-40	21	13	34 (21.79)
41-50	15	08	23 (16.66)
51-60	13	04	17 (10.75)
61-70	11	6	17 (10.75)

A fraction of 59% was identified as positive in direct method of observation by the application of KOH mounting. However, a fraction of 52% was identified as positive in the cultural method. Moreover, few of the cases were common to both the cultural and direct methods. The detail of the distributions is presented (Table 3). The diagnosis was confirmed by microscopic examination (KOH mounting) in 92 cases (59%) that were observed in a detailed process of direct KOH mounting and the causal agents were isolated in culture in 82 cases (52%) identified with the detailed process of cultural with applying certain microbial culture method.

Table 3: Dermatophyte test result through direct and culture method

Test result	Through KOH/ direct detection method		
	Frequency n, (%)	Valid %	Commutative %
Positive	92(59%)	59%	59%
Negative	64(41)	41%	100%
Total	156(100%)	100%	
	Through culture method		
	Frequency n, (%)	Valid %	Commutative %
Positive	82(52%)	52.6%	52.6
Negative	74(47.4)	47.4%	100%
Total	156(100%)	100%	

From the total isolates out of the total positive cases by cultural method a fraction of 17.9% of T. mentagrophytes were the highest among all dermatophytes. The other fractional distribution of dermatophytes isolated were *T.rubrum* 17(11%), *T.tonsurans* 9(6%), *T.violaceum* 9(6%), *E.floccosum* 9(6%), *M. audouinii* 9(6%), *M.gypseum* 5(3.2%), *T. tonsurans* 9(5.8%), *M.canis* 7(4.5%) were among the positive cases and dermatophytic organism isolated. However, a fraction of 35.3% were tested negative (Table 4).

Table 4: Frequency distribution of different dermatophytes

Identified dermatophytes	Frequency	Percent (%)	Valid Percent (%)	Cumulative Percent (%)
T.rubrum	17	10.9	10.9	46.2
T. mentagrophytes	28	17.9	17.9	64.1
T.tonsurans	9	5.8	5.8	69.9
T.violaceum	9	5.8	5.8	75.6
E.floccosum	9	5.8	5.8	81.4
M. audouinii	9	5.8	5.8	87.2
M.gypseum	5	3.2	3.2	90.4
T. tonsurans	8	5.1	5.1	95.5
M.canis	7	4.5	4.5	100.0
Tested negative	55	35.3	35.3	35.3
Total	156	100.0	100.0	

Different types of hyphae are observed during the microscope after the organisms were identified in the culture method. The detatils of the isolated organisms were viewed under the microscope and the structure of those identified dermatopytes presented (Figure 3).

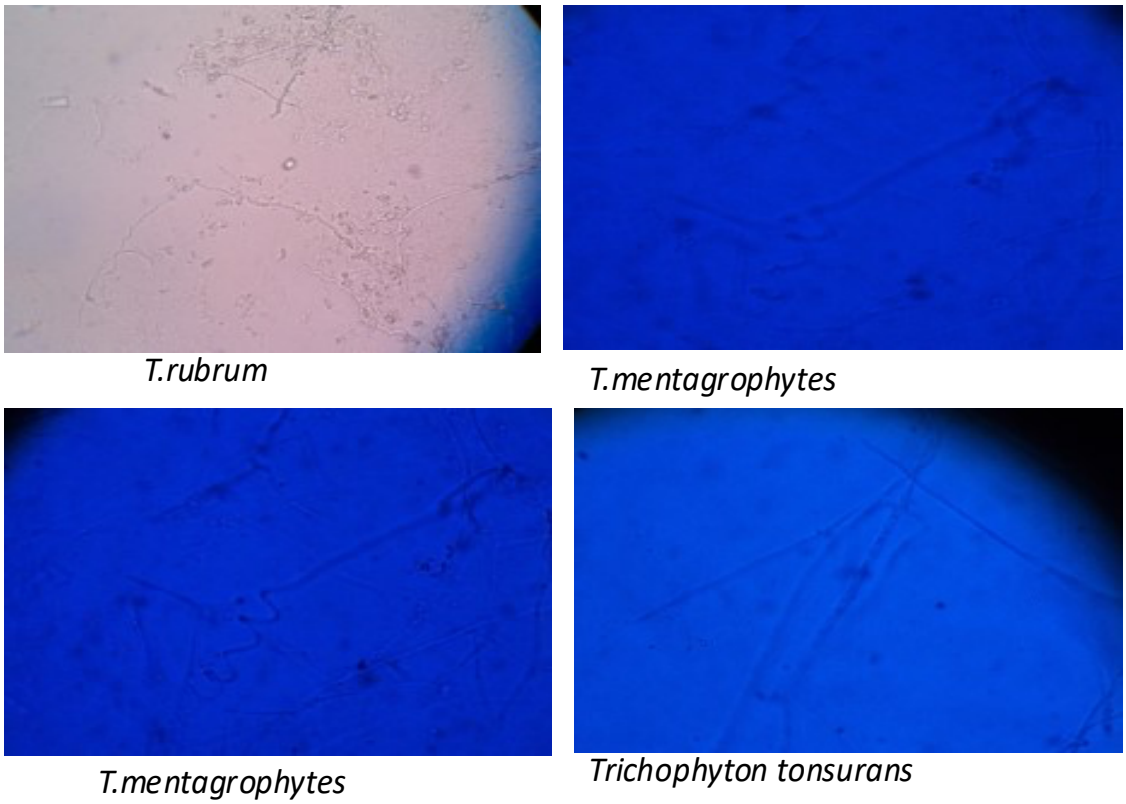

Figure 3: Microscopic view of different organism identified

The final distribution of the diagnosis were T. Corporis 35(22%), T. corporis and T. cruris together with a fraction of 13.5%, individually T. cruris was 10%, and other diagnosis T. unguim 8%, T.pedis 7 %, T. barbae was 6% respectively, a fraction of 33% were the non-identified case in the current observation (Table 5)

Table 5: Frequency distribution of final diagnosis

Final diagnosis	Frequency	Percent	Valid Percent	Cumulative Percent
	52	33.3	33.3	33.3
T. Corporis	35	22.4	22.4	55.8
T.corporis and T. Cruris	21	13.5	13.5	69.2
T.cruris	15	9.6	9.6	78.8
T. Ungium	12	7.7	7.7	86.5
T. Pedis	11	7.1	7.1	93.6
T. Barbae	10	6.4	6.4	100.0
Total	156	100.0	100.0	

Discussion

The incidence of dermatophytic infection is undoubtedly very high. However, neither the medical profession nor the research workers paid any attention to the investigations of these important human infections. In the present studies, a total of 156 cases were enrolled in the study. Among these 73(47%) cases were positive both direct and culturally, and interestingly 59% were positive through mounting of the KOH (direct) method. In the present study, clinically suspected cases of tinea infections were examined over a period of 12 months, among which the most common tinea infections were T. corporis (35 cases), followed by T. corporis and T. cruris together (21, 13.5%), T. ungium (7.7%), T. pedis (7.1%) and T. barbae (6.4 %).

This finding concurred with Omar's 14 results, which emphasized that T. capitis is considered the major type of fungal infection in Egypt. The current findings agreed with those of Achterman and White [4], who reported that—although dermatophytosis is found throughout the world—developed countries have high rates of tinea pedis and onychomycosis, whereas developing countries, like Egypt, have high rates of tinea capitis. In the present study, most of the infections were seen in the younger age group (5–30 years), which may be due to increased physical activity and increased opportunity for exposure. Indeed, Madhavi et al. (2011) [5] found that tinea infections were more common in the 16-

to 45-year-old age group. In the present study, a higher incidence of tinea infection of the capitis variety in particular in school-going children, resulting in increased transmission between them, would be due to increased contact, overcrowding in classrooms (a significant problem in Egypt), a lack of awareness and apathy to personal hygiene, the sharing of personal items, and exposure to soil and even animals on playgrounds.

A higher incidence of dermatophytosis was seen in males than in females, which supports Sumathi et al.'s [6] findings. Male predominance may be due to increased outdoor physical activities and increased sweating, which create a favourable environment for fungal infections, as well as a greater opportunity for exposure to infection than females. The most common genus of dermatophytes detected was Tricophtyton mentagrophytes. However, Jha et al. [7] found that the most common genus was Trichophyton, followed by Epidermophyton. In the present study; M. canis (zoophilic dermatophytes) was the most common isolate (58 cases; 52.7%), followed by M. gypseum (geophilic dermatophytes; 23 cases; 20.9%), T. mentagrophytes (zoophilic dermatophytes; 18 cases; 16.4%), which could be due to patients' interaction with soil and domestic animals, and finally M. audouinii (anthropophilic dermatophytes; 11 cases; 10%). This finding did not agree with that of Kannan et al. [8], who reported that Trichophyton rubrum (73%) was the most common isolate, followed by Trichophyton mentagrophytes (17%), Trichophyton violaceum, and finally Epidermophyton floccosum. The differences between the current study and previous studies stem from variations in patients' environments and lifestyles.

In the current study, zoophilic fungi represent 69.1% of the isolated dermatophytes (M. canis 52.7% and T. mentagrophytes 16.4%). This result did not differ greatly from previously reported findings in Egypt [9], which could be due to the fact that a considerable number of patients (39.1%) were from rural areas and engaged in agricultural occupations. This finding concurred with Emele and Oyeka's [10] findings, which indicated that purely rural agricultural communities, coupled with the associated crowded and unhygienic rural lifestyles and low economic conditions, might have enhanced the spread of the disease among the population in these regions. Although the results of the current study demonstrated a predominance of zoophilic dermatophytes, other categories were also detected, similar to Nweze and Okafor's [11] findings. These researchers concluded that species of dermatophytes causing different types of tineas vary from country to country and also change with time, geography, environment, climate, occupation, and lifestyle. In the present study, aqueous potassium hydroxide (KOH) was used as a clearing agent for the direct demonstration of fungi in skin or hair scrapings [12], but the addition of dimethyl sulphoxide, as described by Rebell and Taplin [13], was found to be a better preparation over plain KOH. The addition of DMSO allows for the rapid clearing of keratin and almost immediate examination of the sample without a warming of slide [14]. It also prevents the rapid drying of the fluid and, thus, is a better option. The findings suggested that a 10% KOH/40% DMSO test can be used for the diagnosis of dermatophytes infections in remote conditions, where a rapid and low-cost diagnosis is required.

However, a few false positive cases were recorded in the current study observed through microscopy however, negative by culture. In addition, false negative cases were recorded: 9.6% were negative by microscopy but positive by culture. This variation could be due to the non-viability of fungal elements in some cases, inadequacy in sampling due to very small lesions, and possible non-reported partial treatment with antifungal electronic physician Page 2565 agents. On the other hand, Tampieri [15] reported that it seems difficult to rely on the results of direct microscopy with KOH to establish the diagnosis of fungal infection as it could not detect the characteristic morphology of the three genera and it lacks sufficient sensitivity, although it is highly efficient as a screening technique before therapy is initiated because of the expense, duration, and potential adverse effects of the treatment. Given that culture is the gold standard for the isolation and identification of dermatophytes, the sensitivity and specificity of the 10% KOH/40% DMSO mount examination in our study were calculated as 88.2% and 76%, respectively, when compared with the reference standard culture results.

This finding concurred with Girgis et al. [16], who found that KOH direct microscopy had a sensitivity of 88% and specificity of 74%. Culture is considered the mainstay of diagnosis because it not only isolates the organism but also allows for the identification of the etiologic agent, thereby allowing treatment to be tailored appropriately. In our study, two media were used for the culture of samples; the Dermasel agar and SDA were supplemented with chloramphenicol and cycloheximide. The two media proved to be technically good, with no statistically significant difference between them ($p<0.05$) for the primary isolation of dermatophytes from clinical samples. However, of much concern was our finding that the culture of dermatophytes on Dermasel agar was more specific with better topography, texture, color, and colony surface and reverse; macroconidia and microconidia are typical for the species when studied microscopically, and less growth of contaminant bacteria and saprophytic fungi is common.

This elevates the importance of Dermasel agar as a selective medium for the isolation of dermatophytes

and, thus, the treatment of infection. There were reports concerning the failure of topical, as well as systemic antimycotic treatment of dermatophyte infections [17], thereby making it essential to evaluate and standardize simple and reproducible in-vitro assays to determine the antifungal activity of various drugs against dermatophytes. Studies evaluating the in-vitro activity of antifungal agents are rare, particularly in filamentous fungi [18]. Moreover, the current study was more in accordance with the previous study from the eastern part of India which had the more likely results obtained. [19]. the result obtained from the study was comparable with the studies from uthansingh et al. 2019 [19] who identified the dermatophytes both direct and cultural method identifying the dermatophytes in skin and Nail lesions.

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