Mycological Analysis of 150 Cases of Dermatophytosis of Skin, Hair and Nail Attending The Outpatient Department of Skin and Venereology

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Keywords: Dermatophytosis, Direct Microscopy, KOH Preparation, LCB Mount, SDA Medium, Tinea Rubrum, Tinea Mentagrophyte.

ABSTRACT

**Background:** Dermatophytes affect millions of people worldwide. Dermatophytosis inflicts a lot of psychosocial trauma. It is not generally appreciated how disabling a skin disease can be since an apparent trivial rash to the observer may be a source of intense discomfort and stigma to the patient.

**Methods:** The present study involved mycological analysis of 150 cases of dermatophytosis attending the OPD of Skin and Venereology, AIMSR, Bathinda during the period of 1st April 2014 to 30th September 2015. Detailed history was taken. Samples of skin, hair and nail were taken depending upon the part affected. Out of the material collected, part of it was used for direct KOH examination and remaining part was used to inoculate SDA medium with antibiotics for culture. Results of KOH preparation and culture, along with relevant history, were noted in Proforma. The observations and data obtained from the study were compiled and analyzed.

**Result:** KOH examination was positive in 93 (62%) cases while culture was positive in 77 cases (51.34%) Overall, Trichophyton was the most common genus 76 samples (98.7%) isolated followed by Epidermophyton 1 sample. Out of the 77 culture positive cases, T. rubrum was the most common isolate in 51 cases (66.23%) followed by T. mentagrophytes in 22 cases (28.57%).

**Conclusion:** It was concluded that KOH examination gives more positive results as compared to culture. Trichophyton infection is more common than Epidermophyton. T. rubrum is the most common infective dermatophyte out of all varieties.

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Introduction
Although fungi are worldwide, only few of them are considered pathogenic. The pathogenic fungi may give rise to infections in animals and human beings. Most of the agents cause infection of the superficial layers of the integument and only very few give rise to systemic involvement. Recently there has been an increase in the incidence of fungal infections. This increase may be a result of frequent usage of antibiotics, immunosuppressive drugs and various conditions like organ transplantations, lymphomas, leukemias and human immunodeficiency virus (HIV) infections. [1]

Dermatophytes are a major public health problem in the world today affecting millions of people. The estimated lifetime risk of acquiring a dermatophyte infection is between 10- 20 percent. [2] Dermatophytes, with an emerging life risk of 10-20%, affects millions of people worldwide. [3] Dermatophytes inflicts a lot of psychosocial trauma due to attached social stigma and in case of children, sometimes irritation is so much, that it hampers pupil’s concentration in class, as well as representing a potential source of secondary bacterial infection. It is not generally appreciated how disabling a skin disease can be since an apparent trivial rash to the observer may be a source of intense discomfort and stigma for the patient. [4]

Morphological characteristics of commonly encountered dermatophytes [5]-
1. T. rubrum: Growth on SDA medium shows velvety growth and red pigment is present on reverse side of the slope. Microscopic picture of LCB mount shows tear drop microconidia and few long pencil-shaped macroconidia are present.
2. T. mentagrophytes: Growth on SDA shows white to tan colored, cottony or powdery colonies and variable pigment is present. Microscopic picture of LCB mount shows clusters of microconidia and cigar- shaped macroconidia with terminal rat-tail filaments are present.
3. T. tonsurans: Growth on SDA medium shows cream or yellow colored, powdery colonies with central furrows. Microscopic picture of LCB mount shows abundant microconidia and thick walled irregular macroconidia.
4. T. schoenleinii: Growth on SDA shows smooth, waxy and brown colored colonies. Microscopic picture of LCB mount shows hyphal swellings, favic chandelier and chlamydospores.
5. T. violaceum: Growth on SDA medium very slow growing, waxy colonies and violet to purple pigment is present. Microscopic picture of LCB mount distorted hyphae are present and conidia are rare.
6. M. audouinii: Growth on SDA medium shows velvety, slow growing and brown colored colonies. Microscopic picture of LCB mount shows thick walled chlamydospores are present but conidia are rare and irregular.
7. M. canis: Growth on SDA medium shows cottony colonies and orange pigment is present on reverse side of the slope. Microscopic picture of LCB mount shows abundant thick walled spindle-shaped macroconidia with up to 15 septa.
8. M. gypseum: Growth on SDA medium shows powdery and buff colored colonies. Microscopic picture of LCB mount shows abundant thin walled macroconidia with 4-6 septa are present.

Lab Diagnosis: Superficial fungal infections are generally strongly suspected on clinical grounds, but at times the diagnosis needs to be confirmed by laboratory support. These include direct microscopic examination and culture of selected material from the affected area. [6]

Collection of Sample: Lal et al (1983) [7] cleaned the skin lesion with 70% alcohol and the scales from the active margin of the lesion were scraped with a sterile scalpel and collected in a sterile petridish. If vesicles were present, their domes were snipped off. In the toe clefts, the material is collected with an epilation forceps. Hairs for examination should be plucked. In black dot variety, material obtained by scraping gives better results. In case of nails, clipping should as proximal as possible. Bindu (2002) [8] described that in tinea capitis, infected and lusterless hair along with scales were collected. In tinea unguium nail scrapings, clippings and subungual debris were collected. The specimen was collected on clean sheets of paper which was folded for transportation [6]. Lari et al (2003) [9] suggested that samples should be taken from patients using scalpels, forceps and glass slides that had been washed in ethanol and sterilized with a Bunsen burner.

Processing of Sample

2. Culture: According to Weitzman and Summerbell (1995) [10], culture adjunct to direct microscopy and is essential in all nail infections and all those infections which are to be treated by systemic mediation. In all cases, a medium selective against most non-dermatophyte moulds and bacteria are used. Cycloheximide is incorporated as a semi selective agent to reduce the growth of non-dermatophyte fungi, Sabouraud peptone – glucose agar (Emmon’sn Modification) amended with cycloheximide and chloramphenicol is commonly used [10]. Hair and nail were first cut into small pieces and then inoculated. Inoculation was done by using a scalpel and loop (Patwardhan and Dave, 1999). [15] Wide agar slants prepared in large culture tubes or bottles were used. Because growth was slow, four weeks of inoculation was required. However in a majority of dermatophytes, growth and sporulation occurs in five to ten days. When sporulation and pigment formation occurs, lactophenol cotton blue (LCB) mount of the growth was examined.

For identification of dermatophyte, definition of many of its characteristics was essential like colony appearance, color, texture, topography examination of reverse side of colony, topography and arrangement of spores etc. [6] Singh and Beena (2003) incubated the media at 25°C and 37°C for a minimum period of three weeks. [16]

Aims and objectives
- To study dermatophytes in KOH preparation.
- Identification of various species of dermatophytes by culture, LCB mount and other required tests.
- To find out the prevalence of various species of dermatophytes.

Materials and Methods
The present study was conducted on suspected cases of dermatophytosis attending the Dermatology, Venereology & Leprology department of AIMSR, Bathinda from 1st April 2014 to 30th September 2015. Ethical approval from institutional ethical committee of Adesh University was taken before start of the study. Details of the patient and sample collection was taken as per proforma attached.

Method of collection of Skin sample: Skin lesions were sampled from the erythematous, peripheral, actively growing margins of the lesions. Skin was decontaminated with 70% alcohol to remove surface bacterial contamination. An open, sterile 2” Petri dish was held immediately below the area to be sampled and skin scales were flaked into it by using blunt edge of a sterile surgical blade or microscopic glass slide. Whenever there was little scaling as with lesions of the glabrous skin, cellophane tape strips were used to take adequate material. The cellophane strip was pressed against the lesion, peeled off, and was placed adhesive side down on a clean glass microscopic slide on which a drop of 10% KOH solution has been placed. [17, 18]

Method of collection of Hair sample: In suspected cases of tinea capitis, after cleaning the selected area with spirit, dull lusterless hair and stubs of hairs were chosen and plucked by sterile surgical forceps. Hair stubs were collected by scraping with the blunt edge of the scalpel. The roots of the hair were included. Skin scrapings were also collected from sites where fungal infection of hairs is suspected.

Method of Collection Of Nail Samples: In cases of tinea unguium the hands and feet were washed with soap and water, with emphasis on the nails. After drying, the nails were decontaminated with 70% alcohol. In majority of cases of tinea unguium, the material for examination was also taken from the distal end of the nail because it was not practicable to take deep samples from the proximal advancing edge. Samples were taken from the nail plate, nail bed and subungual region of the nails with sterile scalpel.

Processing of the sample

Skin
1. A small portion of skin scrapings was taken on a clean glass slide. Then 2-3 drops of 10% KOH were added (10% aqueous solution of potassium hydroxide (KOH) was used as a clearing agent).
2. Material was teased with teasing needles.
3. Then clean cover slip was placed on it.
4. Preparation was passed over the flame once or twice to eliminate any bubble which formed during the process.
5. Preparation was kept at room temperature for 30 minutes.
6. Then it was examined under low power of the microscope (10X) for branching and septate hyphae and confirmation was made under 40X of microscope.
**Nail**
1. In case of nail sample, the material was kept overnight in 40% KOH and then teasing was done.
2. Then cover slip was placed on the material.
3. It was examined under low power and then high power of the microscope.
4. Specimen was examined for branching and septate hyphae.

**Hair**
1. Hair was cut into 1 cm size and transferred on the glass slide.
2. A few drops of 10% KOH were put on the sample and covered with the cover slip. Hair was not heated as it is delicate and may lose its structure.
3. The hair was examined under low power of the microscope (×10) after 1 minute and then the hair was examined under high power (×40) for branching and septate hyphae.
4. Hair was also examined for ectothrix and endothrix infections.

**Culture**
Skin, nail and hair samples were inoculated after reducing the size to approximately 1 mm. Material was inoculated onto Sabouraud's dextrose agar plate containing 0.05 mg/ml chloramphenicol, gentamicin 0.2 mg/ml and 0.5 mg/ml cycloheximide.

Chloramphenicol and Gentamicin were added to inhibit the bacterial growth and cycloheximide was added to inhibit the growth of saprophytic fungi. Then plates were incubated at 28°C and were examined daily up to 4 weeks for evidence of growth from the edge of the planted material. If no growth appeared, results were declared negative after 4 weeks of incubation.

**Medium used for culture:** Composition of Sabouraud Dextrose Agar (SDA) Peptone 10 gm, Dextrose 40 gm, Agar 20 gm, Distilled Water 1000 ml, Adjust pH at 5.6 Following antibiotics will be added into SDA while boiling but before autoclaving to make it selective for isolation of fungus- Cycloheximide 500 mg, Chloramphenicol 50 mg, Gentamicin 20 mg

Dissolve cycloheximide in 10 ml acetone then add it to the boiling medium and mix properly. Similarly, dissolve chloramphenicol & gentamicin in 10 ml of 95% alcohol and add to the boiling medium. Then remove from heating and mix it well. Dispense the medium into plates and autoclave it at 121°C for 15 minutes. 20

**Identification of the Dermatophytes:** Growth, if and when appeared, was identified from culture characters, colonial morphology, pigment production on the underside of growth and production of microconidia and macroconidia by making LCB mount.

**Method of LCB mount**
1. One drop of LCB stain was taken on the glass slide.
2. A small amount of growth was taken from the periphery with the help of bent wire.
3. Growth was gently teased using two teasing needles.
4. Cover slip was applied by avoiding air bubbles.
5. Growth was seen under low power microscope and was confirmed under high power for septate hyphae, arrangement of micro conidia and macro conidia.

**Composition and preparation of LCB Stain** Phenol crystals 20g, Lactic acid 20ml, Glycerol 40ml, Distilled water 20 ml, Cotton blue 0.075g. The phenol crystals were dissolved in the liquids by gentle warming and then dye was added.

**Identification points of Dermatophytes:** The dermatophytes species of common occurrence were identified from the following characteristics.

**Trichophyton:** Species of Trichophyton attacked the hair, skin and nails.

**T. rubrum:** In T. rubrum, the cultures were cottony to velvety but in some cases powdery appearance was also seen. Reddish to purple pigmentation developed on the reverse of the colony and diffused to the marginal hyphae. Primary cultures developed numerous micro conidia in clusters and singly along the hyphae, few macro conidia, racquet hyphae were also present.

**T. Mentagrophytes:** The cultures in T. Mentagrophytes were powdery to granular, light buff to rose tan in color. The reverse of the colony was wine to brownish in colour. These cultures were urease positive within 7 days. These cultures developed macro conidia and numerous micro conidia in clusters and singly on the hyphae. To confirm the diagnosis of T. Mentagrophytes growth was inoculated on Christensen’s medium which gave positive urease test within 7 days. The tubes, in which no growth appeared up to 4 weeks, were discarded.

**T. Tonsurans:** T. Tonsurans culture showed various degrees of heaped or sunken central growth with folding of surface. The colonies were velvety to powdery and varied in color from white, cream and sulphur yellow. Microscopically, all varieties showed numerous deep stained clavate micro conidia along the sides of hyphae, sessile or on short sterigmata. The hyphae supporting the micro conidia usually remained unstained in LCB preparation. Macro conidia were seen rarely.
**Epidermophyton:** This genus attacked skin and nails.

**E. Floccosum:** The culture of E. floccosum was characteristically velvety to powdery with central radiating furrows and greenish yellow in color. Microscopically, the only conidia produced were the large clavate, multisepate, smooth and thin walled. Micro conidia were seen in some strains.

**Result**

The present study involved mycological analysis of 150 clinically suspected cases of dermatophytosis of skin, hair and nail attending the outpatient department of Dermatology and Venereology, AIMSR, Bathinda.

Detailed history was taken. Samples of skin, hair and nail were taken depending upon the part affected.

Out of the material collected, part of it was used for direct KOH examination and remaining part was used to inoculate SDA medium for culture to isolate and identify the pathogenic Dermatophytes.

Results of KOH preparation and culture along with relevant history were noted in proformas.

The observations and data made in the present study were compiled and analyzed and are being presented in the following pages in the form of tables and graphs.

**Distribution of Samples Collected According to Site**

In the present study of dermatophytosis, out of total 150 samples collected, 123 were skin samples, 15 were nail samples and 12 were hair samples.

**Distribution of 150 Clinically Suspected Cases of Dermatophytosis According to Results of KOH Examination and Culture**

Out of 150 cases of dermatophytosis, KOH examination was positive for fungal hyphae in total 93 cases (62%) and negative in 57 cases (38%). Culture was positive for dermatophytes in total 77 cases (51.34%) and negative in 73 cases (48.66%). Both KOH examination and culture were positive in 67 cases (44.67%) while both KOH examination and culture were negative in 47 cases (31.33%). 26 cases (17.33%) were KOH positive but culture negative while 10 cases (6.66%) were KOH negative but culture positive.

**Total Number of KOH Positive Samples (Only KOH Positive and Both Koh Positive Culture Positive) from 150 Clinically Suspected Cases of Dermatophytosis**

In the present study, out of 123 skin samples, 15 nail samples and 12 hair samples, the following observations were made.

From skin samples only KOH positive samples were 17 and both KOH and culture positive samples were 60. So, total KOH positive samples were 77.

From nail samples only KOH positive sample was 5 and both KOH and culture positive samples were 4. So, total KOH positive samples were 9.

From hair samples only KOH positive sample was 4 and both KOH and culture positive samples were 3. So, total KOH positive samples were 7.

Total number of KOH positive samples from skin, hair and nail were 93.

**Distribution of Samples in 150 Clinically Suspected Cases of Dermatophytosis According to Culture Positivity (Only Culture Positive and Both Culture & KOH Positive)**

In the present study, out of 123 skin samples, 15 nail samples and 12 hair samples, the following observations were made.

From skin samples only culture positive samples were 5 and both culture and KOH positive samples were 60. So, total culture positive samples were 65.

From nail samples only culture positive sample were 3 and both culture and KOH positive samples were 4. So, total culture positive samples were 7.

From hair samples only culture positive sample were 2 and both culture and KOH positive samples were 3. So, total culture positive samples were 5.

Total number of culture positive samples from skin, hair and nail were 77.

**Distribution of various types of tinea infections in 150 clinically suspected cases of dermatophytosis according to KOH examination and culture results**

**Table 1** shows that in the present study, out of 60 cases of tinea cruris, 37 cases (61.67%) were KOH positive, 31 cases(51.66%) were culture positive, 31 cases(51.66%) were both culture and KOH positive and 23 cases (38.33%) were both KOH and culture negative.

Out of 35 cases of tinea corporis, 23 cases (65.71%) were KOH positive, 19 cases(54.28%) were culture positive, 18 cases(51.42%) were both culture and KOH positive and 11 cases(31.42%) were both KOH and culture negative.

Out of 16 cases of tinea pedis, 11 cases (68.75%) were KOH positive, 9 cases (56.25%) were culture positive, 7
cases (43.75%) were both culture and KOH positive and 3 cases (18.75%) were both KOH and culture negative.

Out of 10 cases of tinea capitis, 6 cases (60%) were KOH positive, 5 cases (50%) were culture positive, 3 cases (30%) were both culture and KOH positive and 2 cases (20%) were both KOH and culture negative.

Out of 15 cases of tinea unguium, 9 cases (60%) were KOH positive, 7 cases (46.66%) were culture positive, 4 cases (26.66%) were both culture and KOH positive and 3 cases (20%) were both KOH and culture negative.

Out of 7 cases of tinea manuum, 3 cases (42.85%) were KOH positive, 3 cases (42.85%) were culture positive, 2 cases (28.57%) were both culture and KOH positive and 3 cases (42.85%) were both KOH and culture negative.

Out of 5 cases of tinea faciei, 3 cases (60%) were KOH positive, 3 cases (60%) were culture positive, 2 cases (40%) were both culture and KOH positive and 1 case (20%) was both KOH and culture negative.

Out of 2 cases of tinea barbae, 1 case (50%) was KOH positive, none was culture positive and 1 case was both KOH and culture negative.

**Distribution of KOH And Culture Positive Samples According to The Age of The Cases**

**TABLE 2** shows that in KOH positivity, age group 21-30 was predominant with 33 samples, out of which 26 were skin samples and 6 were nail samples followed by age group of 31-40 years with 20 samples, in which 18 samples were of skin and 2 samples were of nail, 11-20 years age group with 11 samples in which 10 samples were of skin and 1 sample was of hair. In culture positivity, the most dominant age group was of 21-30 years, with total number of 24 samples in which 21 samples were of skin, 3 samples were of nail followed by 31-40 years age group with 19 samples, in which 16 samples were of skin and 3 samples were of nail.

**Distribution of KOH and Culture Positive Samples According to The Sex Distribution of The Cases**

**TABLE 3** shows that out of total 93 KOH positive samples, 60 samples were of male patients and 33 samples were of female patients.

In 60 samples of male patients, 52 KOH positive samples were of skin, 4 samples of nail and 4 samples were of hair.

In 33 samples of female patients, 25 KOH positive samples were of skin, 5 samples were of nail and 3 were of hair.

In 77 culture positive samples, 50 samples were of male patients and 27 samples were of female patients.

In 50 samples of male patients, 44 culture positive samples were of skin, 2 samples were of nail and 4 samples were of hair.

In 27 samples of female patients, 21 culture positive samples were of skin, 5 samples were of nail and 1 sample was of hair.

So in our study we see that male patient samples show more KOH and culture positivity than female patient samples.

**Distribution of KOH and Culture Positive Samples According to The Population Staying in Rural and Urban Areas**

**TABLE 4** shows that out of total 93 KOH positive samples, 41 samples were taken from rural population out of which 32 were skin samples, 4 were nail samples and 5 samples were of hair. In the urban population there were 52 KOH positive samples, out of which 45 were skin samples, 5 were nail samples and 2 were hair samples. In 77 culture positive samples, 35 samples were from rural population, out of which 29 samples were of skin, 3 samples were of nail and hair each. In urban population total culture positive samples were 42, out of which 36 samples were of skin, 4 samples were of nail and 2 samples were of hair.

**Distribution of KOH and Culture Positive Samples According to Occupation**

**TABLE 5** shows that among the 93 KOH positive cases, the most affected profession was of farmers with 34 samples followed by labourers with 20 samples, students with 11 samples, housewives with 10 samples, sportsman with 8 samples, business class with 6 samples, armyman and service class with 2 samples each.

In case of culture positivity farmers formed the most predominant group with 27 samples followed by labourers with 16 samples, housewives with 10 samples. Students with 9 samples, sportsman with 6 samples each, business class with 4 samples, army man with 3 samples and service class with 2 samples.

**Species of Fungus Isolated from 77 Culture Positive Cases of Dermatophytosis**

Out of 77 culture positive cases, *T. rubrum* was the most common isolate 51 samples (66.23%) followed by *T. mentagrophytes* 22 samples (28.57%), *T. tonsurans* 3 samples (3.89%), *E. floccosum* 1 sample (1.29%).

Overall, *Trichophyton* was the most common genus 76 samples (98.7%) isolated followed by *Epidermophyton* 1 sample (1.29%).
Table 1: Distribution of Various Types of Tinea Infections in 150 Clinically Suspected Cases of Dermatophytosis According to KOH Examination And Culture Results

<table>
<thead>
<tr>
<th>Type of Tinea</th>
<th>KOH positive Culture negative</th>
<th>KOH positive Culture positive</th>
<th>KOH negative Culture positive</th>
<th>KOH negative Culture negative</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Tinea cruris</td>
<td>6</td>
<td>31</td>
<td>0</td>
<td>23</td>
<td>60</td>
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<tr>
<td>Tinea corporis</td>
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<td>1</td>
<td>11</td>
<td>35</td>
</tr>
<tr>
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<td>7</td>
<td>2</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Tinea capitis</td>
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<td>3</td>
<td>2</td>
<td>2</td>
<td>10</td>
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<tr>
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<td>4</td>
<td>3</td>
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<td>15</td>
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<td>1</td>
<td>3</td>
<td>7</td>
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<td>1</td>
<td>1</td>
<td>5</td>
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<td>Tinea barbae</td>
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<td>0</td>
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<td>TOTAL</td>
<td>26</td>
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Table 2: Distribution of KOH and Culture Positive Samples According to The Age of The Cases

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<tr>
<th>Age group (in years)</th>
<th>SKIN</th>
<th>NAIL</th>
<th>HAIR</th>
<th>TOTAL</th>
<th>CULTURE POSITIVE</th>
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<tbody>
<tr>
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<tr>
<td>11-20</td>
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<td>11</td>
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<td>21-30</td>
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<td>31-40</td>
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<td>20</td>
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<td>41-50</td>
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<td>7</td>
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Table 3: Distribution of KOH and Culture Positive Samples According to The Sex Distribution of The Cases

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<tr>
<th>Sex</th>
<th>SKIN</th>
<th>NAIL</th>
<th>HAIR</th>
<th>TOTAL</th>
<th>CULTURE POSITIVE</th>
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<td>4</td>
<td>60</td>
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<tr>
<td>FEMALE</td>
<td>25</td>
<td>5</td>
<td>3</td>
<td>33</td>
<td>21</td>
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<tr>
<td>TOTAL</td>
<td>77</td>
<td>9</td>
<td>7</td>
<td>93</td>
<td>65</td>
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Table 4: Distribution of KOH and Culture Positive Samples According to The Population Staying in Rural and Urban Areas

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<tr>
<th>Sex</th>
<th>SKIN</th>
<th>NAIL</th>
<th>HAIR</th>
<th>TOTAL</th>
<th>CULTURE POSITIVE</th>
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</thead>
<tbody>
<tr>
<td>MALE</td>
<td>52</td>
<td>4</td>
<td>4</td>
<td>60</td>
<td>44</td>
</tr>
<tr>
<td>FEMALE</td>
<td>25</td>
<td>5</td>
<td>3</td>
<td>33</td>
<td>21</td>
</tr>
<tr>
<td>TOTAL</td>
<td>77</td>
<td>9</td>
<td>7</td>
<td>93</td>
<td>65</td>
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Table 5: Distribution of KOH and Culture Positive Samples According to Occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>SKIN</th>
<th>NAIL</th>
<th>HAIR</th>
<th>TOTAL</th>
<th>Culture positive</th>
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<td>Farmers</td>
<td>32</td>
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<td>1</td>
<td>34</td>
<td>27</td>
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<tr>
<td>Labourers</td>
<td>18</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Housewives</td>
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<td>3</td>
<td>-</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Students</td>
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<td>-</td>
<td>5</td>
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<td>5</td>
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<tr>
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<tr>
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<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Service class</td>
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<td>-</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

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T. rubrum predominated as the causative agent of dermatophytosis in the present study.

Species of Fungus Isolated from Culture Positive Cases of Various Types of Dermatophytosis

TABLE 6 shows that out of 60 cases of tinea cruris, 31 samples were culture positive and T. rubrum was the isolated in 25 cases (80.64%) and T. mentagrophytes was isolated in 6 cases (19.35%). T. rubrum was predominant species in tinea cruris cases.

Out of 35 cases of tinea corporis, 19 cases (54.28%) were culture positive and T. rubrum was isolated in 11 cases (57.89%), T. mentagrophytes was isolated in 7 cases (36.84%) and T. tonsurans was isolated in 1 case (5.26%). T. rubrum was predominant species in tinea corporis cases.

Out of 10 cases of tinea capitis, 5 cases (50%) were culture positive and T. rubrum, T. mentagrophytes were isolated in 2 cases (40%) each and T. tonsurans was isolated in 1 case (20).

Out of 16 cases of tinea pedis, 9 cases (56.25%) were culture positive and T. rubrum was causative agent in 5 cases (55.56%), T. mentagrophytes positive in 3 cases (33.33%) and T. tonsurans was positive in 1 case (11.11%). T. rubrum and T. mentagrophytes were predominant species in tinea pedis cases.

Out of 15 cases of tinea unguium, 7 cases (46.67%) were culture positive and T. mentagrophytes and T. rubrum were isolated 3 cases (42.85%) each and E. floccosum was isolated in 1 case (14.28%).

Out of 7 cases of tinea manuum, 3 cases (42.86%) were culture positive and T. rubrum was isolated in 2 cases (66.66%) and T. mentagrophytes was isolated in 1 case (33.33%).

Out of 5 cases of tinea faciei, 3 cases (60%) were culture positive. T. rubrum was the only species isolated.

Various Species of Fungus Isolated from Skin, Hair and Nail Samples in 150 Clinically Suspected Cases of Dermatophytosis

In present study, out of 77 culture positive samples T. rubrum was isolated from 51 samples followed by T. mentagrophyte isolated from 22 samples, T. tonsurans from 3 samples and E. floccosum isolated from 1 sample.

Thus, in this study T. rubrum was the prominent dermatophyte species from all the sites.

Various Species of Fungus Isolated from Positive Skin/Hair/Nail Samples According to Age Group

In the present study, out of 65 culture positive skin samples, 21 samples were in 21-30 years age group out of which

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<table>
<thead>
<tr>
<th>CLINICAL SITE</th>
<th>DERMATOPHYTE SP</th>
<th>Culture positive Only</th>
<th>KOH &amp; Culture both positive</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. cruris</td>
<td>T. rubrum</td>
<td>-</td>
<td>25</td>
<td>25</td>
<td>80.64%</td>
</tr>
<tr>
<td></td>
<td>T. mentagrophytes</td>
<td>-</td>
<td>6</td>
<td>6</td>
<td>19.35%</td>
</tr>
<tr>
<td>T. corporis</td>
<td>T. rubrum</td>
<td>-</td>
<td>11</td>
<td>11</td>
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<tr>
<td></td>
<td>T. mentagrophytes</td>
<td>-</td>
<td>7</td>
<td>7</td>
<td>36.84%</td>
</tr>
<tr>
<td></td>
<td>T. tonsurans</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>5.26%</td>
</tr>
<tr>
<td>T. capitis</td>
<td>T. rubrum</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>T. mentagrophytes</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>T. tonsurans</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>T. pedis</td>
<td>T. rubrum</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>55.56%</td>
</tr>
<tr>
<td></td>
<td>T. mentagrophytes</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>33.33%</td>
</tr>
<tr>
<td></td>
<td>T. tonsurans</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>11.11%</td>
</tr>
<tr>
<td>T. unguinum</td>
<td>T. rubrum</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>42.85%</td>
</tr>
<tr>
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<td>T. mentagrophytes</td>
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<td>2</td>
<td>3</td>
<td>42.85%</td>
</tr>
<tr>
<td></td>
<td>E. floccosum</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>14.28%</td>
</tr>
<tr>
<td>T. manuum</td>
<td>T. rubrum</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>66.66%</td>
</tr>
<tr>
<td></td>
<td>T. mentagrophytes</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>33.33%</td>
</tr>
<tr>
<td>T. faciei</td>
<td>T. rubrum</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>100%</td>
</tr>
</tbody>
</table>
15 samples were of T. rubrum followed by 5 samples of T. mentagrophytes and 1 sample of T. tonsurans. In 31-40 years age group out of 16 culture positive samples 10 samples were of T. rubrum followed by 6 samples of T. mentagrophytes.

Similarly in other age groups also, T. rubrum was the predominant causative agent of dermatophytosis.

In the present study, out of 5 culture positive hair samples, 0-10 years age group shows 4 culture positive samples in which T. mentagrophyte was isolated in 2 samples and T. rubrum was isolated in 1 sample. In 11-20 years age group 1 sample was culture positive from which T. rubrum was isolated.

Thus T. rubrum and T. mentagrophyte were the most common isolates in hair samples.

In the present study, out of 7 culture positive nail samples, 21-30 years age group shows 3 culture positive samples and T. mentagrophytes was isolated from 2 samples and E. floccosum was isolated from 1 sample. In 31-40 years age group from 3 culture positive sample T. rubrum was isolated from 2 samples and T. mentagrophyte was isolated from 1 sample.

Thus T. rubrum and T. mentagrophyte were the most common isolates in nail samples.

**Various Species of Fungus Isolated from Culture Positive Skin/Hair/Nail Samples in Males and Females**

In the present study, out of 44 culture positive skin samples in male patients, T. rubrum was isolated in 33 samples followed by 11 samples of T. mentagrophytes. Out of 21 culture positive skin samples in female patients, T. rubrum was isolated in 13 samples followed by 6 samples of T. mentagrophytes and 2 samples of T. tonsurans. In both male and female patients, T. rubrum was predominant dermatophyte species in culture positive skin samples.

In the present study, out of 4 culture positive hair samples in male patients, 2 samples were of T. rubrum and 1 sample each from T. mentagrophytes and T. tonsurans. Out of 1 culture positive hair sample in female patient, T. mentagrophytes was isolated.

In the present study, out of 2 culture positive nail samples in male patients, T. rubrum and T. mentagrophyte were isolated from 1 sample each. Out of 5 culture positive nail samples in female patients, T. rubrum was isolated in 2 samples, T. mentagrophyte in 2 samples followed by E. floccosum in 1 sample. Thus, T. rubrum was predominant dermatophyte species in culture positive nail samples.

**Discussion**

The present study involved mycological analysis of 150 cases of dermatophytosis of skin, hair and nail attending the outpatient department of Skin and Venereology, AIMSR, Bathinda. Detailed history was taken. Samples of skin, hair and nail were taken depending upon the part affected. Out of the material collected, part of it was used for direct KOH examination and remaining part was used...
to inoculate SDA medium for culture. Results of KOH preparation and culture, along with relevant history, were noted in Proforma. The observations and data obtained from the study were compiled and analyzed.

**Mycological Observations**

**Results of 150 clinically suspected cases which were under mycological study**

**KOH positivity in 150 clinically suspected cases**

In the present study, out of 123 skin samples, 15 nail samples and 12 hair samples, the following observations were made.

From skin samples only KOH positive samples were 17 and KOH and culture positive samples were 60. So the total KOH positive samples were 77.

From nail samples only KOH positive sample were 5 and KOH and culture positive samples were 4. So the total KOH positive samples were 9.

From hair samples only KOH positive samples were 4 and KOH and culture positive samples were and KOH and culture positive samples were 3. So the total KOH positive samples were 7.

Total number of KOH positive samples from skin, hair and nail were 93.

In the present study, out of the 150 cases examined, 93(62%) were positive for fungal hyphae on direct microscopy of KOH preparation while 57(38%) samples were KOH negative. The KOH positivity was higher in skin samples at 77 cases (62.6%) overall while it was 7 cases (58.33%) in hair samples and 9 cases (60%) in nail samples.

**Culture positivity in 150 clinically diagnosed cases**

The culture was positive in 77(51.33%) samples and negative in 73(48.67%) samples. The culture positivity in skin samples was 65 samples (52.84%), in nail samples it was 7 samples (46.66%) and in hair samples it was 5 samples (41.66%).

Out of the 150 cases, both KOH and culture were positive in 67 cases while both KOH and culture were negative in 47 cases. 26 cases were KOH positive culture negative while 10 cases were culture positive and KOH negative.

From skin samples only culture positive samples were 5 and culture and KOH positive samples were 60. So the total culture positive samples were 65.

From nail samples only culture positive sample were 3 and culture and KOH positive samples were 4. So the total culture positive samples were 7.

From hair samples only culture positive sample were 2 and culture and KOH positive samples were 3. So the total culture positive samples were 5. KOH and culture positivity rates have a wide range in different studies.

Poria et al (1981) [19] observed a KOH positivity rate of 44.3% and culture was positive in 37.6% samples.

Sharma et al (1983) [20] reported that out of total 114 cases, 101 cases (88.6%) were KOH positive and out of these, 48 gave positive cultures. Of the 13 KOH negative cases, 4 were positive in culture, in all, 52 cultures were positive giving positivity rate of 45.6%.

Jain et al (2008) [21] reported that out of 120 cases of dermatophytosis, 87 cases (72.5%) were KOH positive and 70 cases (58.3%) were culture positive.

Sarma and Borthakur (2007) [22] found that out of 100 cases 90 were KOH positive and 61 were culture positive.

Clearly all the studies are showing higher KOH positivity as compared to culture positivity. These findings are in accordance with the present study.

**Various species of dermatophytes isolated**

In the present study, 77 samples were positive on culture. In all four species of dermatophytes were isolated. T. rubrum was the most common isolate at 66.23 % and the second commonest species, T. mentagrophytes was far behind at 28.57%. Next came T. tonsurans at 3.9% and E.floccosum at 1.29%. Overall, Trichophyton was the most common genus at 98.7% followed by Epidermophyton (1.29%). No Micosporum was isolated.

Present study clearly showed that T. rubrum was predominant in skin samples as the causative agent in 46 samples followed by T. mentagrophytes in 17 samples, T. tonsurans in 2 samples. T.rubrum & T.mentagrophyte were predominant in nail samples as the causative agent in 3 samples each followed by E. floccosum in 1 sample. T.rubrum & T.mentagrophyte were predominant in hair samples as the causative agent in 2 samples each followed by T. tonsurans in 1 sample.

Clearly T. rubrum with 51 samples was predominated in the study followed by T. mentagrophytes in 22 samples, T. tonsurans in 3 samples and E. floccosum in 1 sample.

T. rubrum was isolated in all clinical types of tinea and was the most common isolate in all forms

Various authors have found T. rubrum to be the most common isolate.

Poria et al (1981) [19] reported T. rubrum in 44% cases followed by T. mentagrophytes (18.6%), T. violaceum (7.6%), E. floccosum (4.2%) and T. tonsurans (0.8%).
Lal et al (1983) [7] also found T. rubrum to be the commonest pathogen isolated at 57% followed by T. mentagrophytes (31.2%), E. floccosum (6.5%) and T. violaceum (3.9%).

Khare et al (1985) [12] also observed T. rubrum as the commonest dermatophyte (78.4%) in their study followed by T. mentagrophytes (12.4%), E. floccosum (6%) and T. violaceum (3.2%). These findings are well comparable with our study.

Trichophyton genus was the commonest (98.7%) in our study.

This was also observed by Weitzman et al (1998) [10], who reported that Trichophyton remains the most frequently isolated genus, exceeding by far combined genera of Epidermophyton and Microsporum.

**Tinea cruris:** In our study from 60 cases of tinea cruris, 6 were KOH positive culture negative and 31 were both KOH and culture positive. Out of 31 culture positive cases, T. rubrum constituted 25 cases (80.64%) which were maximum, T. mentagrophytes 6 cases (19.35%). Weitzman and Summerbell (1995) [10] reported T. rubrum, E. floccosum and T. mentagrophytes are commonly associated with Tinea cruris.

**Tinea corporis:** In our study from 35 cases of tinea corporis, 5 were KOH positive culture negative, 1 was culture positive KOH negative and 18 were both KOH and culture positive. Out of 19 culture positive cases, T. rubrum constituted 11 cases (57.89%) which was maximum, T. mentagrophytes 7 cases (36.84%), T. tonsurans 1 case (5.26%). According to Weitzman and Summerbell (1995) [10], T. rubrum, E. floccosum and T. mentagrophytes are commonly associated with tinea corporis. Prasad et al (2005) [23] reported that in tinea corporis, T. rubrum is most commonly implicated in 17.3% cases.

**Tinea pedis:** In our study from 16 cases of tinea pedis, 4 samples were KOH positive culture negative and 2 samples were culture positive KOH negative. 7 samples were both KOH and culture positive. Out of 9 culture positive cases, T. rubrum constituted 5 (55.56%) cases which were maximum, T. mentagrophytes 3 cases (33.33%) and T. tonsurans 1 case (11.11%). According to Weitzman and Summerbell (1995) [10], T. rubrum and T. mentagrophytes are commonly associated with tinea pedis.

**Tinea capitis:** In our study from 10 cases of tine capitis, 3 samples were KOH positive culture negative, 2 samples were culture positive KOH negative and 3 were both KOH and culture positive. Out of 6 KOH positive hair samples, 4 (66.66%) were endothrix type while 2 (33.33%) were ectothrix type. Out of 5 culture positive cases, T. rubrum and T. mentagrophytes constituted 2 cases (40%) each while T. tonsurans constituted 1 case (20%). In a study on tinea capitis, 88% had endothrix while 12% had ectothrix spores (Sehgal et al, 1985). [11] Kalla et al (1995) [24] observed endothrix spores in 78% cases while ectothrix was seen in 22% which is closer to our observation of endothrix (66.66%) and ectothrix (33.33%).

**Tinea unguium:** In our study from 15 cases of tine unguium, 5 were KOH positive culture negative, 3 were culture positive KOH negative and 4 were both KOH and culture positive. Out of 7 culture positive cases, T. rubrum and T. mentagrophytes constituted 3 cases (42.85%) each and E. floccosum 1 case (14.28%). According to Weitzman and Summerbell (1995) [10], T. rubrum, E. floccosum and T. mentagrophytes are commonly associated with tinea unguium, tinea corporis and tinea pedis while in tinea unguium and tinea manuum, T. rubrum and T. mentagrophytes are often isolated.

Brillowska et al (2007) [25] found in his study on 118 cases of onychomycosis that T. rubrum was the major pathogen.

**Tinea manuum:** In our study from 7 cases of tine manuum, 1 case was KOH positive culture negative, 1 case was culture positive KOH negative and 2 were both KOH and culture positive. Out of 3 culture positive cases, T. rubrum constituted 2 cases (66.66%) and T. mentagrophytes constituted 1 case (33.33%) only. According to Weitzman and Summerbell (1995) [10] in tinea manuum, T. rubrum and T. mentagrophytes are often isolated.

**Tinea faciei:** In our study from 5 cases of tine faciei, 1 was KOH positive culture negative, 1 was culture positive KOH negative and 2 were both KOH and culture positive. T. rubrum was isolated in all three culture positive samples (100%).

**Tinea barbae:** In our study from 2 cases of tine barbae, 1 was KOH positive culture negative and 1 was both KOH and culture negative.

**Positive KOH and culture samples according to age distribution**

Positive KOH and culture samples according to age distribution shows that in KOH positivity, age group 21-30 year was predominant with 33 samples (35.48%), out of which 26 were skin samples and 6 were nail samples followed by age group of 31-40 years with 20 samples (21.50%), 18 samples of skin and 2 of nail. 11-20 years age group with 11 samples (11.82%), 10 samples of skin and 1 of hair followed by other age groups.

In culture positivity, the most dominant age group was of 21-30 years, with total number of 24 samples (31.16%) followed
by 31-40 years age group with 19 samples (24.67%). The least common age group were 61-70 & 71-80 years with 2 sample (2.59%) each. In culture positive cases T. rubrum dominated.

Singh and Beena (2003) [16] also found in their study that 45.5% of their patients with positive samples were in the 16-30 years age group.

Mahmoudabadi (2005) [26] observed that 35.7% of the cases with positive samples were in the 21-30 years age group.

Positive KOH and culture samples according to sex distribution

In the present study male: female ratio was 1.78:1. Out of total 93 KOH positive samples, 60 (64.51%) were of males and 33 (35.48%) were of females.

In 77 culture positive samples, 50 (64.93%) were of males and 27 (35.06%) were of females.

Clearly our study shows that males dominated KOH and culture positivity in skin and hair, while females dominated KOH and culture positivity in nail samples. Males associated with agriculture and farming class are more exposed to moist conditions and trauma which predisposes to infection, especially during paddy season.

In culture positive cases T. rubrum dominated in both sexes.

Patwardan and Dave (1999) [15] have observed a male to female ratio of 2:1 in cases of dermatophytosis.

Singh and Beena, (2003) [16], noted male: female ratio as 1.57:1.

Positive KOH and culture samples according to occupation

In our study we had noticed that farmers and labourers were predominantly affected followed by housewives. In our country though major part of population stays in villages but in our study only 66 cases (44%) were from rural areas. Persons associated with agriculture showed high positivity for fungal infection that could be attributed to their profession, because they frequently came in contact with soil in the fields, their hands and feet remained moist because they water the fields which predispose to the infection. Secondly housewives were more affected because they wash utensils, clothes and their hands and feet are in contact with water most of the time which predispose to the infection. In case of KOH positive cases, the urban population dominated over the rural population in skin and nail samples, while in hair samples rural population dominated. Same was in the case of culture.

Conclusion

To conclude, the present study of 150 cases of dermatophytosis at AIMS, Bathinda shows that:

The KOH positivity rate was 62% and culture positivity rate was 51.33%. So, KOH examination gives more positive results as compared to culture.

Trichophyton infection is more common than Epidermophyton.

T. rubrum was the most common isolate followed by T. mentagrophyte.

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Competing Interests

None Declared

References