

PREVALENCE STUDY ON SPECIES IDENTIFICATION OF CUTANEOUS DERMATOPHYTOSIS

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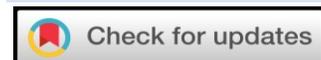
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ABSTRACT

The aim of the study was to provide an evidence-based data on the prevalence of the most common fungal infection, cutaneous dermatophytosis. The study had two arms one clinical part consisting of survey of 80 participants who are clinically diagnosed as cutaneous dermatophytosis. From these survey contestants skin scrapings were taken and undergone fungal positive test that is KOH mount. Only those cases where KOH mount positive is included in the study. As a second part the positive skin scrapings were cultured and after inoculation, they were identified under 40x microscope. Finally, after the study 2 genus's and 6 different species were obtained. Under trichophyton genus comes the 4 species *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. verrucosum* and among *Microsporum* genus *M. canis* and *M. aoudonii*. In this article the identification features of these species externally and microscopically after culturing is to be highlighted along with that prevalence percentage also noted.

Keywords: Cutaneous dermatophytosis, Genus trichophyton, Genus microsporum

INTRODUCTION

Cutaneous dermatophytosis are the common tinea infections which affect the cutaneous tissues of our body. The characteristics of this infections are that; they are

superficial dermatophyte infections characterized by either inflammatory or non-inflammatory lesions of the

glabrous skin. The rate of this disease occurring is increasing globally. For providing a specific diagnosis rather than a vague diagnosis like fungal infections certain specific tests are needed. So, the KOH test is mandatory for diagnosing a fungal infection positive. For specific diagnosis the need of fungal culturing arises. After the growth, inoculation of the specimen under microscope and features were noted. Thus, we got special characteristic features for these different species.

Materials and Methods

Inclusion Criteria

- KOH mount positive
- Age group 16 – 60 years, both sexes irrespective of caste, religion and economic status
- Those who are willing to give consent

Skin scraping examination for fungal Dermatophytes was carried out for confirmation of diagnosis. All the patients were subjected to this examination and registered, if found to be positive for the presence of fungal mycelium or pseudo mycelium. Skin scraping examination. This was carried out in Toxicological lab, Department of Agad Tantra, VPSV Ayurveda College Kottakkal. In this procedure, the skin of the patients from affected area was scraped with a blunt scalpel (no: 20). Scales were placed on center of microscope slide, swept into a small pile, and covered with a cover slip. Capillary action draws solution under cover slip. The preparation was gently heated with a match or lighter until bubbles begin to expand, clarifying the preparation. Excess KOH solution was blotted out. Then stained with Lactophenol cotton blue, slide was then examined by direct microscopy for the presence of fungal hyphae.^[1]

Exclusion Criteria

- Other cutaneous lesions associated with Dermatophytosis
- Patients undergoing other systemic and topical application
- Immuno-compromised patients
- Vulnerable group
- Bleeding disorders

Research techniques and tools

A Case record form was made to record the details of the case. Consent form in Malayalam language was prepared and prior consent of all the participants were obtained on the consent form. A pamphlet containing the details of the research was given to the participants. The whole plan of study was approved by Institutional Ethics Committee (IEC) prior to starting of work (IEC NO:-IEC/CL/02/17 Dated on 27/04/2017) and an interim report on the status of research was also got approved in due course.

Clinical study: The method used is cross-sectional survey

Experimental study

Scrapings will be taken from each sample and mounted with KOH and followed by Lactophenol cotton blue staining to identify the microorganism

- Culture of fungi will be done using SDA agar
- For isolation and identification slide culture, BCP agar, urease test will be conducted
- Phytochemical screening and HPTLC will be done for *Dineśavallitvak cūrṇa extract*
- Antifungal activity against identified species by well diffusion method
- MIC determination by serial agar dilution plate technique

Results

Table 1: Clinical Study: On cross sectional study of the identified species among 80 participants

Serial no	Species name	Number	Percentage
1	Trichophyton rubrum	25	31.25%
2	Trichophyton mentagrophytes	18	22.50%
3	Trichophyton tonsurans	7	8.75%
4	Trichophyton verrucosum	3	3.75%
5	Microsporum canis	1	1.25%
6	Microsporum audouinii	3	3.75%

7	Malazzesia furfur	8	10%
8	Mucor	6	7.5%
9	Yeast	9	11.25%



Fig 1: Colonies of TRM

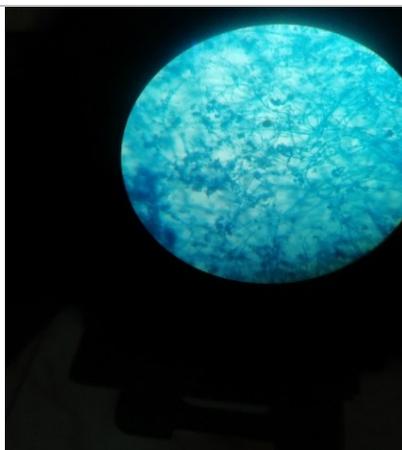
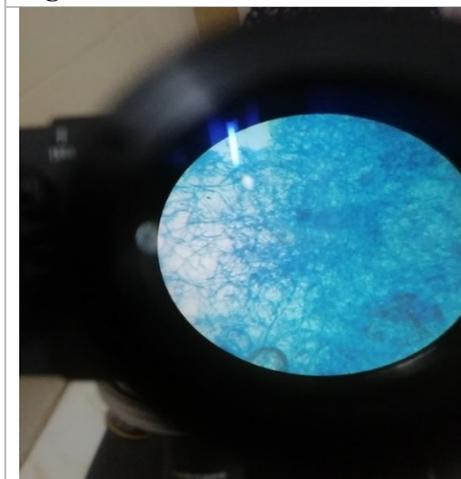


Fig 2: TRM microscopic features



Fig 3: Colonies of TRR



FN 4: TRR microscopic features



FN 5: Micro conidia of TRT



FN 6: Micro conidia of TRR

Identification features

Fungal culturing using SDA Agar

It is the most direct and conclusive methods for diagnosing the fungal infections. Samples were collected and processed through centrifugation, softening or liquidation method. Common media used is SDA Agar. [2]

SDA Agar comprised of enzymatic digest of casein and animal tissues. It provides a nutritious source of amino acids and nitrogenous compounds for the growth of fungi and yeasts.

Table 2: Composition of media

Ingredients	Gms/liter
Mycological peptone	10
Dextrose	40
Agar	15
pH adjusted to 5.6 at 25°C	

Procedure of preparation of media

1. Suspend 65gm of media in 1 liter of distilled water.
2. Heat with frequent agitation and boiled for 1 minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes
4. Cool to 45-50°C and pour in to petri dishes [2]

Process of culturing

After confirming that skin scrapings are positive for fungus using KOH mounting and staining with lacto phenol cotton blue the remaining scrapings are spread on petri dishes containing media. After 3 weeks’ fungal growth was obtained. For processing of specimens,

streak the specimen on to the medium with a sterile inoculating loop in order to obtain isolated colonies. Incubate the plates at 25-30°C in an inverted position (agar side up) with increased humidity. [3]

Identification of genus and species

Based on growth characters’ rapidity

1. Rapidity of growth
2. Color
3. Morphology of the colony

Morphology studied in teased mounts or slide cultures

1. Hyphae diameter
2. Presence /absence of septa
3. Conidia morphology – diagnostic importance

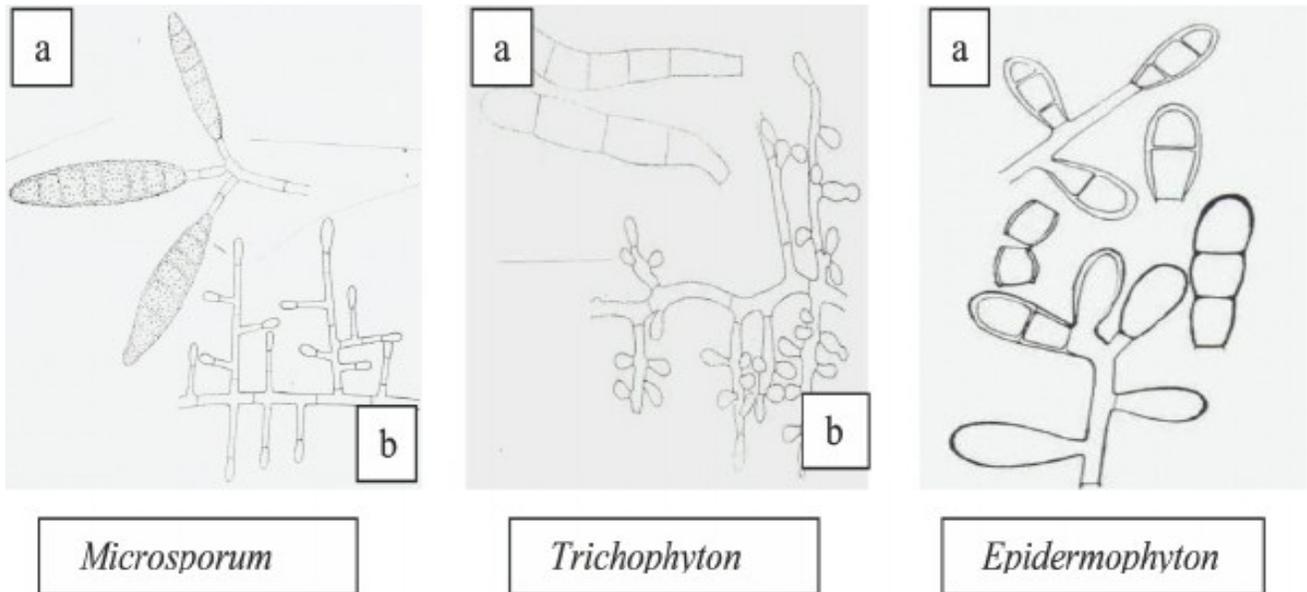


Fig 7: Picture of three genera of dermatophyte species [4]

In lesions Dermatophytes appear as hyphae and arthrospores. In cultures of sabouraud’s agar, they form characteristic colonies consisting of septate hyphae and two types of asexual spores, micro conidia and macro conidia. Differentiation in to the three genera is based mainly on the nature of macro conidia.

Trichophyton: Colonies may be powdery, velvety or waxy, with pigmentation characteristic of different species. Micro conidia are abundant and are arranged in clusters along the hyphae or borne on conidiophores. Macro conidia are relatively scanty. They are generally

elongated, with blunt ends. Macro conidia have distinctive shapes in different species and are of importance in species identification. Some species possess special hyphal characters, such as spiral hyphae, racquet mycelium and favic chandeliers. Trichophyton infect skin, hair and nails. Trichophyton rubrum is the most common species infecting human beings. It often causes chronic, treatment resistant lesions. In Trichophyton mentagrophytes colony will be seen as white to tan colored and its texture will be like cottony or powdery appearance and its morphology is cigar shaped rat tail

end. Another species is *Trichophyton tonsurans* in this type colony will be like cream or yellow colored with central furrows and irregular macro conidia with thick wall is seen. In *Trichophyton schoeleinii* colony will be smooth, waxy, and brownish the morphology will be seen like hyphal swelling, chlamydospores, favic chandelier. In *Trichophyton violaceum* very slow growing. Waxy, violet to purple pigment colony will be there and distorted hyphae and rare conidia are found. [5]

Microsporum

Colonies are cotton-like, velvety or powdery, with white to brown pigmentation. Micro conidia are relatively scanty and are not distinctive. Macro conidia are the predominant spore form. They are large, multicellular, spindle shaped structures, borne singly on the ends of hyphae. *Microsporum* species infect the hair and skin but usually not the nails. In *Microsporum audouinii* species velvety, brownish, slow growing colonies are seen and on morphology thick walled chlamydospores, conidia rare and irregular. *Microsporum canis* species colony will be seen like cottony, orange pigment on reverse and on morphology abundant, thick walled, spindle shaped macro conidia with up to 15 septa are seen. *Microsporum gypseum* is another species here colony will be powdery and buff colored. On morphology abundant, thin walled macro conidia with 4-6 septa. [5]

DISCUSSION

Here cross-sectional study was conducted to check the prevalence of causative agents that are causing cutaneous dermatophytosis in Kottakkal population. 80 was the sample size the patients who are clinically diagnosed with Tinea infection, the scrapings are taken and after doing the KOH test and seeing that it is positive that sample is included in the study. After that for identifying its species the remaining scrapings were spread on SDA agar plate, after 2 weeks' fungal colonies were obtained on the SDA plate and they are observed under compound microscope. By analyzing the colony characters and characteristics obtained in compound microscope the species were identified. In this way more than 100 samples were collected and identified 80 species by the method mentioned above. Among the species

identified more prevalent were *Trichophyton rubrum* 31% and *Trichophyton mentagrophytes* 22%.

CONCLUSION

Cutaneous Dermatophytosis is nothing but the Tinea infections predominantly characterized by an annular reddish pink patch with elevated peripheral margin with slight scaling leading to severe itching caused by fungus. On prevalence study in Kottakkal population the prevalence was found to be different as that of reported values. *Microsporum* species was noted in our population. *Trichophyton* species was same as that of the reported values.

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