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Research article

A comparative analysis of fungal species isolated from skin, nail and hair of patients attending dermatology OPD

Suganthi M*, Thenmozhivalli, Thyagarajan Ravinder, Selvi, Dillirani, Anand
Department of Microbiology, Government Kilpauk Medical College, Chennai, Tamilnadu, India

Abstract

The skins and related infections it can be occur very commonly in general population. Usually among all other kind of microbes the fungal is effective to attack on skin. The common cause of skin infections are dermatophytes and opportunistic fungi. Aim of this study was to isolate and identify the fungal species from clinical samples from patients. Clinical samples from 170 patients were subjected to potassium hydroxide (KOH) examination and culture isolation; causative agents were identified microscopically. Out of 170 specimens, 42 specimens were both KOH and culture positive. Dermatophytes were isolated in 60 specimens. T. rubrum was the most common isolate. The study identified different dermatophytes in skin, nail and hair.

Keywords: Fungal infection, skin infection, nails and hair, dermatophytes

***Corresponding author: Dr. Suganthi M,** Department of Microbiology, Government Kilpau Medical College, Chennai, Tamilnadu, India. E- mail: suganlalith@gmail.com

1. Introduction

The cutaneous infection of human includes a wide variety of diseases in which the integuments and its appendages, the hair and the nail are involved. Infection is generally restricted to the non-lining cornified layer but a variety of changes occur in the host because of the presence of the infectious agent and its metabolic products. Majority of the infections are caused by a homogenous group of keratophilic fungus called the dermatophytes. A single species might be involved in several clinical types each with its distinct pathology. [1] The fungi are the commonest infective agent of human and no group of people or geographical areas are without taenia or ringworm infection (taenia-latin for worm). Evolutionary development towards an accommodating host parasite relationship can be seen among the dermatophytes which is absent among other fungal agent of human disease. This group of disease is collectively referred to as dermatophytosis.

Dermatophytes are a group of closely related group of organisms that can use keratin as a nitrogen source.[2] On the basis of clinical, morphologic and microscopic characteristics three genera are recognized as dermatophytes; Trichophyton, Microsporum and Epidermophyton. Depending on their natural habitat dermatophytes may be anthropophilic (people loving), zoophilic (animal loving) and geophilic (soil loving).Dermatophytes are not endogenous pathogens. Transmission of dematophytes occurs via three sources, each resulting in typical features. Table 1 shows types of dermatophtes based on mode of transmission and their clinical manifestations.

Dermatophyte includes several distinct clinical varieties, depending on the anatomical site and the etiological agent involved. The pathology induced on the host initially is an eczemiform response followed by allergy and inflammatory manifestations. The type and severity of these reactions are related to the immune status of

the host as well as to the strain and species of the organism causing the infection.

Dermatophytosis remains a significant public health problem. Numerous anti-fungal agents have been developed since Grisofluvin became available through a breakthrough experimental work of Gentles in guinea pig in 1958. This discovery revolutionized the therapeutic approach to this disease. In 1980 discovery of azoles' and derivatives and allied groups of antifungal drugs had significant impact in the management of dermatophytosis. The increasing number of immunosupressed patients and expanding drug resistance of microorganisms make the development and appropriate use of the most important areas in microbiology and infective medicine.
In present study, we thought to isolate and identify different fungal species from skin disease patient from a regional medical Centre.

2. Patients and methods

It was a prospective cohort study of patients who attended the mycology section in the Dermatology outpatient department at Stanley Medical College and hospital, Chennai, India between May 2008 and Jun 2009. A total of 170 patients were enrolled in the study after fulfilling inclusion criteria of clinically diagnosed dermatophytosis and not undergoing treatment for the diagnosis. The study was approved by institutional ethics committee and all the enrolled patients provided consent form. The patients who were receiving treatment for the diagnosis were excluded from the study.

Collection of Samples

All the patients were asked to provide relevant details and skin scrapping; nail clippings and hair were collected after the patients' approval. The samples were collected in dark paper sachets for transport to laboratory to easily visualize the scales. These papers were sterilized in autoclave for 15 minutes at 121°C.

Skin

The skin specimen was collected as described previously. [3] Briefly, the affected area was thoroughly swabbed with 70% alcohol followed by drying by evaporation. Then, the active edge of the lesion was scrapped with a flame sterilized blunt scalpel without any injury to the skin.

Scalp

The same procedure was followed as described above; in addition, a few affected hair were also epilated and collected with a pair of flame sterilized tweezers. Care was taken to collect the basal portion of the hair as fungus is usually found in this area.

Nail

Briefly, nail clippings taken from the discolored, dystrophic or brittle parts of the nails. The affected nail was meticulously swabbed with 70% alcohol. After which the nail was clipped or scrapped deeply enough to obtain recently invaded nail tissue.[4]

Processing of specimen

Isolated were identified by earlier suggested method. Briefly, a small amount of specimen was placed on a glass slide with 1-2 drops of10-20% KOH solution with warming over a low flame, which hastens the digestion of keratin. A drop of LPCB (Lacto Phenol Cotton Blue) was also added for better visualization of hyphae. Hyphal elements were examined under low power and high power objectives of light microscope. The ring worm fungi was differentiated from epidermal cell outlines , elastic,cotton and vegetable fibres and artifacts such as intra cellular cholesterol.

All the samples collected were inoculated on to Sabourauds Dextose agar (Emmon's modification; SDA) containing chloramphenicol (50mg/L) and cyclohexamide (500 mg/L) and into a second tube of SDA with gentamycin to detect the growth of other non-dermatophytes in the clinical sample. The slopes were incubated at 25˚C and examined at intervals for evidence of fungal growth. Slopes not showing growth for 4 weeks were discarded. The isolates were inoculated on to Potatoe dextrose agar for better consideration, the isolates were also inoculated on to Blood agar slopes and modified Christensen's medium for differentiation of species.

Fungal slide culture

The slide culture is used to study undisturbed morphological details particularly relationship between reproductive structures like conidia, conidiophore and hyphae. Fungal slide culture was performed in cases with doubtful morphology.[5]

Briefly, a sterile microscope slide was placed on a bent glass rod at the bottom of a petri dish. A piece of one square centimeter block of Sabouraud dextrose agar or potato dextrose agar was put up on the slide. The fungal strain under identification at four sides of agar block was inoculated and incubated at 25˚C in BOD incubator. A drop of LCB on a slide and coverslip from block was placed upon growth. The slide culture was examined the details microscopically to identify the fungus. The mycelia which adhere to the glass surface usually show characteristic microscopic appearance which may be lost if teasing needle are used as happens in the routine LCB mounts. The slide culture was also directly examined by putting under low power of microscope.

3. Results

Out of the 170 specimens collected; 20 specimens were collected from hair and 75-75 specimens were collected from skin and nail scrapings. Of the 60 culture positive isolates, 42 (70%) were KOH wet mount positive and culture positive, 18 were KOH negative and culture positive and 13 were KOH positive and culture negative. The site-based specimen details have also been shown in table 2.

Table 1. Specimen details

Site	Number of specimen	% Specimen
Total number of specimen	170	-
Skin Scrapping	75	44.11
Nail Scrapping	75	44.11
Hair	20	11.76

Identification of Specific- based dermatophytes

It was observed that Tinea corporis was the major clinical presentation 49(65.3%) in skin (fig 1a), while Tinea capitiswas the major presentation from hair. Tinea unguium was the only presentation from nail clipping (fig 1b). A detailed analysis has been presented in table 3.

Table 2. Clinical types of lesions from different specimens

Specimen	Clinical types of Lesions	Number of Cases	%
Skin Scrappings (n=75)	Tinea corporis	49	65.3
	Tinea cruris	19	25.3
	Tinea mannum	4	5.3
	Tinea pedis	3	4
Hair (n=20)	Tinea capitis	18	90
	Tinea barbae	2	10
Nail Clipping (n=75)	Tinea unguium	75	100

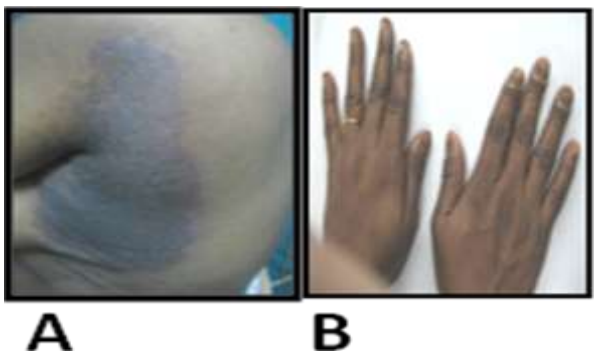

Figure 1 shows clinical presentation of dermatophytes a) Tinea corporis b) Tinea ungium

Dermatophytes Isolated from the Skin

Dermatophytes were identified in a total of 28 skin scrappings. In Tinea corporis, T.rubrum was the predominant isolate 7/16 (35.7%) followed by T.mentagrophytes 4/16(25%), T. tonsurans (12.5%) and M.gypseum 2/16(12.5%). In T. cruris, isolates of 2(33.3%) each of T. verrucosum and E.floccosum, 1(16.6%) Out of 4 dermatophytes isolated from Tinea manum, 2 were from T. mentagrophytes and 2 each of T. rubrum and T. tonsurans. Out of 3 dermatophytes isolated from Tinea pedis, 2 were T. tonsurans and 1was T rubrum. Out of 2 dermatopytes isolated, one each of T.mentagrophytes and T. verrucosum were isolated. A detailed analysis has been presented in table 3.

Table 3.Dermatophytes Spp (Skin)

Dermatophytes Spp	Tinea corporis	Tinea cruris	Tinea mannum	Tinea pedis	Total	%
T.rubrum	7	1	1	1	10	35.7
T.mentagrophytes	4	1	2	-	7	25
T.tonsurans	2	-	1	2	5	17.8
T.verrucosum	-	2	-	-	2	7.1
E.floccosum	-	2	-	-	2	7.1
M.gypseum	2	-	-	-	2	7.1
Total	15	6	4	3	28	

Dermatophytes isolated from nail

Out of the 21 isolated dermatophytes in Tinea unguium, T. rubrum was the predominant isolate followed by T. mentagrophytes. There was 2 isolates of T. tonsurans,4 isolates were T. verrucosum, 2 isolates were T. violateum and 2 isolates were T. schoenlinii (table 4).

Table 4. Dermatophytes (Nail)

Dermatophytes	Number of isolates	%
T.rubrum	6	28.5
T.mentagrophytes	5	23.8
T.tonsurans	2	9.5
T.verrucosum	4	19
T.violateum	2	9.5
T.schonlienii	2	9.5
Total	21	

Dermatophytes isolated from hair

Out of 11 dermatopytes isolated of Tinea capitis, 4 were T.violaceum and 4 were T.tonsurans, T. verrucosum and M. audoiuni. 2isolates from T.barbae, one each of T.mentagrophytes and T. verrucosum (table 5).

Table 5. Dermatophytes (Hair)

Dermatophytes	Tinea capitis	Tinea barbae	Total	%
T.menagrophytes	-	1	1	9
T.tonsurans	3	-	3	11.1
T.verrucosum	1	1	2	27.2
T.violateum	4	-	4	36.3
M.audounii	1	-	1	9

4. Discussion

In present study out of the 60 culture positive isolates, 42 (70%) were KOH wet mount positive and culture positive, 18 were KOH negative and culture positive and 13 were KOH positive and culture negative (data published earlier). The present study identified dermatophytes in 60 specimens (28 skin, 21 nail and 11 hair). T. rubrum was the main isolate form skin scraping 35.7% (10/28) in the present study similar to other investigators' reports.[6-8] This is the commonest agent isolated form skin of the body, groin folds and the feet.[9] T. mentagrophytes was the second common isolate from the body site 7/28 (25%) as also shown by.[10] E. floccosum was isolated from two specimens obtained from the skin which remain the other common isolate from the skin scrapping.[10]

T. violaceum seemed to be the chief isolate from the hair 36.3% (4/11). This agent is still the commonest isolate from cases of tinea capitis in India.[6] T. audinii was the least common isolate (1/11) from the hair. The other isolates from the nail specimens T.tonsurans, T. verrucosum, T.barbae, T.mentagrophytes and T.verrucosum.

Conclusion

The present study showscommonest fungi has been observed to be much higher in this study. The present study revealed that the different isolates from skin scrapping, nail and hair. The clinical presentation was different in different specimens.

Reference

[1] Richard K, Scher PK. Onychomycosis: A significant medical disorder. J Am Acad Dermatol 1996:35:S2-5.
[2] Rippon J.W. Medical Mycology, Dermatophytosis and Dermatomycosis, 3rd edition, P 267
[3] Kamalam A. Observation on te Gamut/Galaxy of the fungal infection in Tamilnadu state. Kavaka. 1984;12:15-36
[4] Emmons CW.Binford CH, utz JP AND Kwag Chang KJ(edi) Medical Mycology, 3rd edition P131.
[5] Bailey and Scott, Diagnostic Microbiology 10th edition
[6] Sentamil SG. Chronic dermatophytosis a clinical and aetiopathological study. 1995. Ph. D thesis submitted to University of Madras.
[7] Lim JT, Chua HC, Goh CL. Deramtophyte and non-deramtophyte onychomycosis in Singapore. Aust J Dermatol. 1992;33:159-63
[8] Kannan P, Janaki C, Selvi GS. Prevalence of dermatophytes and other fungal agents isolated from clinical samples. Indian J Med Microbiol. 2006;24(3):212-5
[9] Kamalam A, Thambiah AS. Histological study in Tinea capitis. Mykosen 1981; 24: 431-41
[10] Kamalam A, Thambiah AS. Prevalence of deramtomycoses in Madras city. Indian J Med Res 1981; 73: 513-8