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ORIGINAL ARTICLE

DISTRIBUTION AND CHARACTERIZATION OF DERMATOPHYTES IN A TERTIARY CARE HOSPITAL IN MADHYAPRADESH

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ABSTRACT: Superficial mycosis with dermatophytes has a wide geographical distribution and diverse species prevalence. This mycological study comprised of 204 clinically suspected cases of dermatophytosis. All 204 samples were subjected to direct microscopy by potassium hydroxide (KOH) mount and isolation on Sabouraud's dextrose agar containing chloramphenicol without cycloheximide. Culture confirmation was done by tease mount, slide culture and various physiological tests like urease test, hair perforation test etc. In the present study an agar based disk diffusion method was performed to determine the susceptibility of dermatophytes. In this study 49% cases were positive for fungus in microscopy, while 24% was culture positive. The most common clinical types observed was T. cruris (40%) followed by T. corporis (34.3%). Young adults in the age group of 21-30 years were most commonly affected, belonging to lower middle socio-economic status. Among cultures, *T. rubrum* was the most common isolate (53%) followed by *T. mentagrophyte* (38.7%).

KEYWORDS: Dermatophytosis, Trichophyton, Microsporum, Epidermophyton.

INTRODUCTION: The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (Skin, hair and nails) of humans and other animals to produce an infection. Dermatophytosis, commonly referred to as ringworm is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts.[1]However clinical manifestations in immunocompromised hosts are often atypical and more severe than in immunocompetant one.[2]

The pattern of skin disease in India is affected by various ecological factors like economic background, environmental factors, literacy level, social, mental and racial factors. Fungal infections have attracted the attention of physicians and microbiologist in recent years due to various reasons like indiscriminate use of antibiotics, anticancer therapy and immunodeficiency diseases like AIDS.[3]

There are few published data available in India mainly from southern and north-eastern part[4,5] but there is paucity of reports from central India. The present study is an attempt to determine the distribution and phenotypic characterization of dermatophytosis as well as their susceptibility pattern against commonly used antifungal agents.

MATERIAL AND METHODS: This study was conducted in Department of Microbiology and Dermatology in R. D. Gardi Medical College and C. R. Gardi Hospital and research Centre, Surasa, Ujjain. The study included patients with suspected dermatophytic infections, visiting skin OPD from Jan. 2012 to June 2013. Samples were obtained from the patients in the Dept. of Microbiology. For the study relevant clinical history & informed consent was obtained.

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Before taking the sample affected area was thoroughly cleaned with 70% ethanol. The samples were taken in a sufficient amount from the edge of the infected area, using a sterile scalpel blade. For the lesions having vesicles or bullae, the top of the vesicles or bullae were clipped and included in the sample.

In case of onychomycosis nail clipping were collected in addition to nail scrapings from the lesions whenever it was feasible. Hair roots and crusts were plucked from the infected area so that the basal portion of hair (hair stub) is included, as the fungus was usually found in this area.

The collected specimens were divided into two portions. The first portion of the specimen was examined microscopically using 10% KOH for skin scrapings and 20% KOH for hair & nails for the presence of fungal hyphae and/or arthrospores. The second portion was cultured on Sabouraud's dextrose agar containing chloramphenicol (0.05%) without cycloheximide and was incubated at 25°C for 4 to 6 weeks. Cultures were examined twice a week. If no growth was obtained till 6 weeks they were declared negative.

Clinical isolates were identified on the basis of phenotypic characteristics of the colonies (morphology, rate of growth and production of pigmentation), microscopic examination of lactophenol cotton blue tease mounts and slide culture. Additional physiological tests such as urease production, in vitro hair perforation tests were performed to differentiate different species as per standard procedure.[6]

In the present study an agar based disk diffusion method was performed to determine the susceptibility of dermatophytes. A total of 15 dermatophytes strains, including *Trichophyton rubrum* (n =9) and *T. mentagrophytes* (n = 6) were tested. The fungi were maintained in culture tubes according to Clinical Laboratory Standards Institute (CLSI) guidelines. Fluconazole (25 µg/disk), Ketoconazole (10 µg/disk), Clotrimazole (10µg/disk) and Itraconazole (10µg/disk) obtained from Hi media Mumbai were used.

Preparation of inoculum suspensions was based mainly on the National Committee for Clinical Laboratory Standards (NCCLS) guideline.[7] Organisms were sub cultured on Potato dextrose agar (PDA) at 30°C for 4 to 15 days to enhance conidial growth. Mueller-Hinton (MH) agar plates were streaked evenly with a cotton swab dipped into the standardized inoculum suspension in a four different direction (at 90^0 angle) to cover the entire surface. Disks containing the antifungal agents were applied to the surfaces of inoculated plates. Plates were inverted and incubated at 30°C for 4 to 7 days to allow for fungal growth. Inhibition zone diameters (IZD) were measured in millimeters with a scale after complete fungal growth. Two readings were taken at right angle. The average of two readings was recorded.

All isolates were run in duplicates. Candida albicans American Type Culture Collection (ATCC) 90028 served as quality control (QC) strains. Its zone size was within the acceptable range recommended by the CLSI. The interpretation of antifungal susceptibility testing was followed according to criteria attempted and published by other researchers.[8,9]

RESULTS: The most common clinical type observed was T. cruris (40.0%) followed by T.corporis (34.3%) and the least of T. pedis (2.12%) [Table-1]. Of the 204 samples included in the study 100 (49%) were KOH positive, of which 43(43%) were culture positive also. The remaining 104(51%) samples were KOH negative, out of which 6 (6%) were positive for fungal culture. Thus in present study, culture positivity is 24% (49 cases) [Table-2]. Overall male to female ratio was 3.25:1. The

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most common age group affected was 21-30 years [Table-3]. *T. rubrum* was the most common isolate (53%) followed by *T. mentagrophyte* (38.7%), *T. verrucosum* (6.12%) and only one isolate of *E. floccosum* (2%) were found [Table-4]. According to Kuppuswami's classification of socio economic status[10] most of the patients fall in lower middle (38.2%) and upper lower (30%) class. Dermatophytic infections were least seen in upper socio economic status (6.86%) [Table-5].

In the present study, an agar based disk diffusion method was performed to determine the susceptibility of dermatophytes. All fifteen strains were sensitive to itraconazole, ketoconazole & clotrimazole. Only one strain of *T. rubrum* and *T. mentagrophyte* was resistant to fluconazole. [Table-6].

Sr. no.	Clinical type	No. of cases (%)
1	Tinea cruris	82 (40)
2	Tinea corporis	70 (34.3)
3	Tinea unguium	29 (14.2)
4	Tinea capitis	08 (4.0)
5	Tinea manuum	06 (3.0)
6	Tinea barbae	05 (2.45)
7	Tinea pedis	04 (2.12)

Table 1: Clinical types of Dermatophytosis in present study (n=204)

KOH mount	Culture positive (%)	Culture negative (%)
KOH positive (n=100)	43 (43)	57 (57)
KOH negative(n=104)	6 (5.8)	98 (94.2)

Table 2: Correlation between KOH mount and culture (n=204)

Age (yrs) Sex Clinical types	0-10 M/F	11-20 M/F	21-30 M/F	31-40 M/F	41-50 M/F	51-60 M/F	61-70 M/F	TOTAL M/F
Tinea cruris	1/0	14/3	17/7	15/5	9/2	4/1	3/1	63/19
Tinea corporis	2/1	5/1	14/2	12/4	8/4	8/2	5/2	54/16
Tinea unguium	-/-	1/1	7/3	8/2	4/1	1/-	1/-	22/7
Tinea capitis	3/2	1/2	-/-	-/-	-/-	-/-	-/-	4/4
Tinea manuum	-/-	1/-	1/1	1/1	1/-	-/-	-/-	4/2
Tinea barbae	-/-	-/-	1/-	1/-	1/-	1/-	1/-	5/2
Tinea pedis	-/-	2/-	1/-	-/-	1/-	-/-	-/-	4/-
Total	**6/3**	**24/7**	**41/13**	**37/12**	**24/7**	**14/3**	**10/3**	**156/48**

Table 3: Dermatophytosis in relation to Age and Sex (n=204)

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Clinical types Isolates	*T. cruris*	*T. corporis*	*T. barbae*	*T. capitis*	*T. pedis*	*T. Manum*	*T. unguim*	Total (%)
T. rubrum	16	9	-	-	1	-	-	26 (53)
T. mentagrophyte	07	10	01	-	-	01	-	19 (38.7)
T. verrucosum	01	01	-	-	-	-	01	03 (6.12)
E. floccosum	01	-	-	-	-	-	-	01 (2.0)
Total	**25**	**20**	**01**	**-**	**01**	**01**	**01**	**49**

Table 4: Species of Dermatophytes isolated from different clinical types (n=49)

Socio-economic status	No. of cases (%)
Upper	14 (6.86 %)
Upper middle	33 (16.2 %)
Lower middle	78 (38.2 %)
Upper lower	61 (30%)
Lower	18 (8.8 %)

Table 5: Relationship of dermatophytic infections with socio-economic status of the patients

Isolates	Sensitive	Resistant
***T. rubrum* (n=9)**		
Itraconazole	9	-
Fluconazole	8	1
Clotrimazole	9	-
Ketoconazole	9	-
***T. Mentagrophyte*(n=6)**		
Itraconazole	6	-
Fluconazole	5	1
Clotrimazole	6	-
Ketoconazole	6	-

Table 6: Interpretation of antifungal susceptibility testing (n=15)

DISCUSSION: In present study T. cruris was the commonest presentation (40%) followed by T. corporis (34.3%). High incidences of these two types were also seen in various studies.[11,12] Clinically KOH positivity as a primary tool for identification of fungal elements in the clinical sample showed positivity in 49% which is consistent with other reports.[13] Maximum number of cases of dermatophytosis belonged to the age-group 21-30 years (26.47%) followed by age-group 31-40 years (24%) similar inference has been drawn by other workers also.[14] The higher incidence may be due to increased physical activity leading to increased sweating in this age group. Male to female ratio in present study was 3.25:1 which is in concordance with other studies.[13,15] Higher prevalence in

males as compared to females may be due to more physical activity, more awareness and tight clothing worn by males. *T. rubrum* (53%) was most common etiological agent followed by *T. mentagrophyte*, which is comparable with the study of other authers.[16]

In present study most of the patients fall in lower middle (38.2%) and upper lower (30%) class. This trend was attributed may be due to prevalence of poor hygienic practices, poverty and lack of education as well as misconceptions about disease and social belief which leads to seek non-medical advice and remedies. Moreover, only severe and chronic infections compelled the patients to attend the hospitals, as was also reported by previous workers from various parts of India.[17,18]

In consonance and conformity with other similar studies, the antifungal susceptibility testing by disk diffusion method was found to be simple, reliable, reproducible and provides an effective alternative for use in routine clinical laboratory.[19,20]

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