American Journal of Psychiatric Rehabilitation Expert Opinion Article

Isolation And Identification Of Fungi Causing Dermatophytosis

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ABSTRACT

INTRODUCTION: Dermatophytes are the most important fungi affecting the skin due to their extensive impact on people across the globe and their high prevalence in the general population.[1] This study aims to isolate and identify the dermatophytes and to compare the clinical diagnosis with potassium-hydroxide smear positivity and culture positivity.

MATERIALS & METHODS: 75 clinically suspected patients of dermatophytosis who attended the Department of Dermatology were included in the study after giving informed consent. The specimen is mounted in KOH (10% for skin scrapings and hair, 20-40% for nail clippings) and was examined for the presence of thin, septate, and hyaline hyphae. The samples were inoculated on SDA slants containing 0.05% chloramphenicol, 0.1% gentamicin, and 0.5% Cycloheximide and dermatophyte test medium(DTM).

RESULTS: The samples were collected from a wide range of age group falling in the range of 6-78 years. Most common age group affected was found to be 31-40 years. 22.85% cases of Tinea corporis were seen in the age group of 21-30 years. Tinea cruris was more common in the age group 21-30 and 31-40 years each with 4 cases(23.52%). 50% of children were affected with Tinea capitis .

CONCLUSION: The infection predominantly affects individuals between 31-40 years of age, with a higher incidence in males, especially those engaged in manual labor or household work. Laboratory identification through microscopy and culture confirms that *T. rubrum* is the most common species involved, followed by *T. mentagrophytes* and *T. tonsurans*. This highlights the need for targeted preventive measures and effective treatment strategies in these high-risk groups.

KEY WORDS: Dermatophytosis , *T. mentagrophytes,* Microscopy

INTRODUCTION

Fungi are now recognized as significant causes of morbidity and mortality among man and animals. Dermatophytes are the most important fungi affecting the skin due to their extensive impact on people across the globe and their high prevalence in the general population.[1] Dermatophytes are closely related to keratinophilic fungi with the ability to degrade keratin and invade the skin, hair, and nails, thus causing dermatophytosis.[2] Dermatophytes are assuming greater significance in developed and developing countries particularly due to the advent of immunosuppressive drugs and disease.[3] Hot and humid climates in tropical and subtropical countries like India make dermatophytosis or ringworm a widespread superficial fungal skin infection.[3,4,5] Species distribution and prevalence vary with the geographical area and over time and are governed by environmental conditions, personal hygiene, and individual susceptibility.

The epidemiology of most of the clinically significant dermatophytosis has substantially changed over the last few years.[1,6] The clinical presentation is very often confused with other skin disorders particularly due to the rampant application of broad-spectrum steroids, making laboratory diagnosis and confirmation necessary and although it responds to conventional antifungals, dermatophytosis tends to recur at the same or different sites. [3,7] Correct diagnosis is important to initiate appropriate treatment and is also essential for epidemiological purposes. Therefore, a study of dermatophytosis in a population is very important as it may reflect the climatic conditions, hygienic, and socio-economic status of the people.[8]

This study aims to isolate and identify the dermatophytes and to compare the clinical diagnosis with potassium-hydroxide smear positivity and culture positivity.

MATERIALS & METHODS

The study was conducted in the Department of Microbiology from June 2012 to July 2014 at IMS & SUM Hospital, Odisha after due clearance from the

Institutional Ethical Committee with code IMS SH/IEC/2014/64.

Inclusion Criteria:
All clinically suspected cases of dermatophytosis.

Exclusion Criteria:
Patients who were on treatment for fungal infection.

Study Population :
75 clinically suspected patients of dermatophytosis who attended the Department of Dermatology were included in the study after giving informed consent. A detailed history of each patient was recorded concerning name, age, gender, address, occupation, duration of illness, and site of involvement**.** Clinical examination of the patient was made in good light which included the site of lesion, number of lesions, and presence of inflammatory margin.

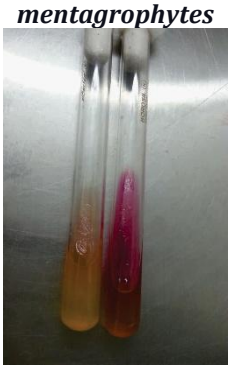
Specimen Collection, Processing and Identification
The specimens like skin scales, crusts, pieces of nails, and hairs were collected. The specimen was mounted in KOH (10% for skin scrapings and hair, 20-40% for nail clippings) and was examined for the presence of thin, septate, and hyaline hyphae. The samples were inoculated onto SDA slants containing 0.05% chloramphenicol, 0.1% gentamicin, and 0.5% Cycloheximide and dermatophyte test medium(DTM). The slants were incubated at 25^0C in BOD and inspected every day for four weeks for the appearance of visible growth of the fungal colony.

The isolates were checked based on rapidity and type of growth on DTM. Colony morphology was studied and microscopic features and other characteristics were correlated for identification of the species. The shape, texture, colour, consistency, wrinkling, and dryness of the colony on the obverse side were noted. (**Fig-1)** The colonies were teased using Lactophenol cotton blue mount and checked for the presence of hyphae and spores.

Identification of dermatophytes was done based on macroscopic and microscopic features. Apart from culture, other methods like hair perforation test, and urease test were done to differentiate between *T. mentagrophytes and T. rubrum*. (**Fig-2)**

Fig-1 : DTM with growth

Fig-2 : Urease Test positive for *T. mentagrophytes*



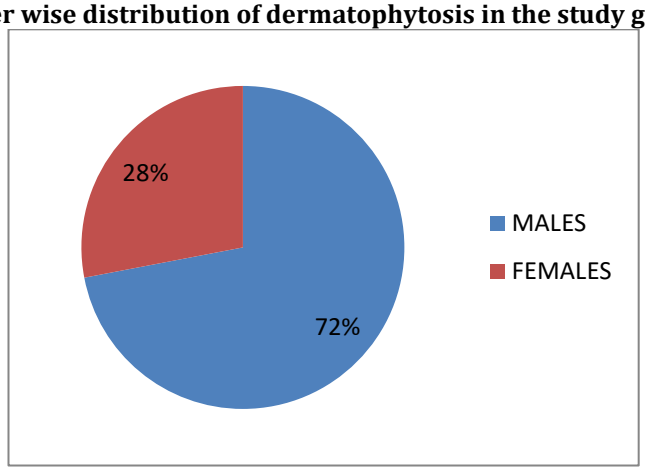
RESULTS
During the study period from June 2012 to July 2014, a total of 75 samples were included in the study . The samples were collected from a wide range of age group falling in the range of of 6-78 years. Most common age group affected was found to be 31-40 years with 18 cases (24%) followed by 21-30 years with 16 cases (21.33%) and 41-50 years with 12 cases (16%). Least common age group affected was ≤ 10 years with 3 cases (4%) followed by >70 years with 4 cases (5.33%). (**Table-1)**

Table 1: Age wise distribution of dermatophytosis in the study group (n=75)

Age (years)	Males	Females	Percentage% (No. of cases)
≤ 10	3.50%(2)	5.56%(1)	4%(3)
11-20	10.53%(6)	11.11%(2)	10.66%(8)
21-30	21.05%(12)	22.22%(4)	21.33%(16)
31-40	22.80% (13)	27.78%(5)	24%(18)
41-50	17.55%(10)	11.11%(2)	16%(12)
51-60	10.53%(6)	11.11%(2)	10.66%(8)
61-70	8.79%(5)	5.56%(1)	8%(6)
>70	5.27%(3)	5.56%(1)	5.33%(4)
Total	100%(57)	100%(18)	100%(75)

Out of 75 cases, males were more commonly affected with 57 cases (76%) than females with 18 cases (24%). (**Fig-3)**

Fig 3: Gender wise distribution of dermatophytosis in the study group (n=75)



8(22.85%) cases of Tinea corporis were seen in the age group of 21-30 years. Tinea cruris was more common in the age group 21-30 and 31-40 years each with 4 cases(23.52%). 50% of children were affected with Tinea capitis . (**Table-2)**

Table-2: Age wise distribution in relation to clinical types

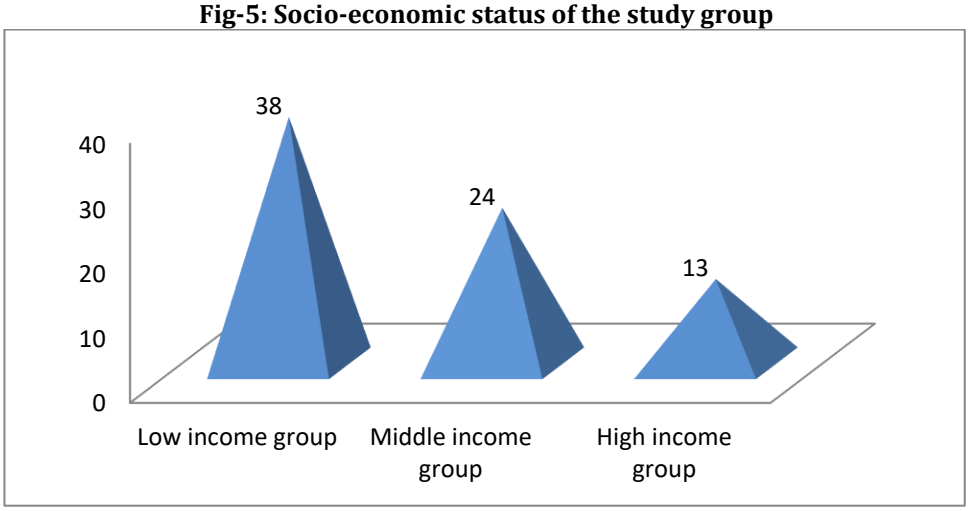
Age	Tinea corporis	Tinea cruris	Tinea unguium	Tinea capitis	Tinea pedis	Tinea faciei	Tinea manuum
<10	0	0	0	3(50%)	0	0	0
11-20	3(8.5%)	2(11.7%)	2(16.6%)	1(16.6%)	0	0	0
21-30	8(22.85%)	4(23.5%)	2(16.6%)	1(16.6%)	0	1(50%)	0
31-40	7(20%)	4(23.5%)	4(33%)	1(16.6%)	1(50%)	0	1(100%)
41-50	6(17%)	3(17.6%)	2(16.6%)	0	0	1(50%)	0
51-60	6(17%)	2(11%)	0	0	0	0	0
61-70	3(8.5%)	1(5.8%)	1(8.3%)	0	1(50%)	0	0
>70	2(5.7%)	1(5.8%)	1(8.3%)	0	0	0	0
TOTAL	35	17	12	6	2	2	1

Out of 75 cases , 24(68.57%) males and 11 (31.42%) females were diagnosed with Tinea corporis. **(Fig-4)** 14 males (82.35%) were identified as Tinea cruris . Tinea unguium was more common in males with 8 cases (66.67%).

Fig-4: Tinea corporis

Majority of the cases were from low income group with 38 cases (50.66%) followed by middle income group with 24 cases (32%) and high income group with 13 cases (17.33%). (**Fig-5)**

Fig-5: Socio-economic status of the study group



Out of 75 cases, most common clinical type was tinea corporis with 35 cases(46.66 %) followed by tinea cruris (22.66%), tinea unguium (16%), tinea capitis (8%), tinea pedis (2.67%), tinea faciei (2.67%) and tinea manuum (1.34%).Tinea corporis was most commonly seen in manual workers with 18 cases (51.42 %) followed by household workers with 10 cases (28.57%). (**Table-3)**

Table-3: Various clinical types in relation to occupation

CLINICAL TYPE	MANUAL	HOUSEHOLD	STUDENT	OTHER	TOTAL
Tinea corporis	18(51.42%)	10(28.57%)	5(14.28%)	2(5%)	35(46.66%)
Tinea cruris	12(70%)	4(23.52%)	1(5%)	-	17(22.66%)
Tinea unguium	6(50%)	4(33.33%)	1(8%)	1(8%)	12(16%)
Tinea capitis	1(16.66%)	-	4(66.66%)	1(16.66%)	6(8%)
Tinea pedis	1(50%)	1(50%)	-	-	2(2.6%)
Tinea faciei	1(50%)	-	1(50%)	-	2(2.6%)
Tinea manuum	1(50%)	-	-	-	1(1.33%)
TOTAL	40(53.33%)	19(25.33%)	12(16%)	4(5.33%)	75(100%)

Out of 75 clinically suspected cases of dermatophytosis, fungi were demonstrated in 52 cases (69.34%) either by direct microscopy and/or culture.20 cases (38.46%) were positive by both microscopy and culture. 30 (57.6%) were positive by microscopy and negative by culture. 2 cases (3.8%) were negative by microscopy but culture positive. (**Fig-6, Table-4)**

Fig-6: KOH Mount- showing fungal elements

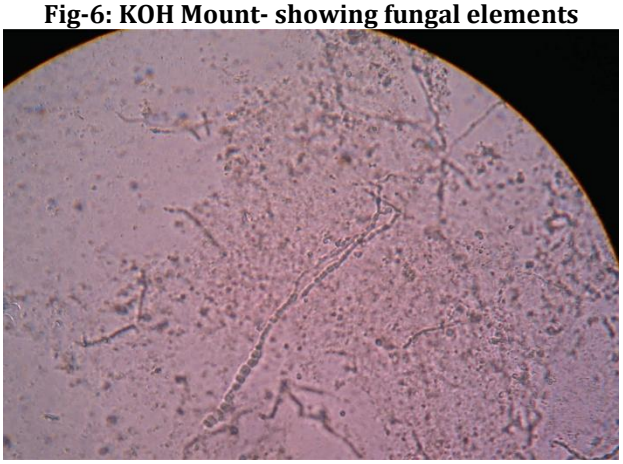


Table-4: KOH and culture findings

	Positive by KOH and/orculture +ve	KOH +ve Culture +ve	KOH +ve Culture -ve	KOH –ve Culture +ve
NO.OF CASES	52 (69.34%)	20(38.46%)	30(57.6%)	2(3.8%)
Total	75(100%)	75(100%)	75(100%)	75(100%)

In tinea corporis, out of 8 culture isolates, T rubrum was the commonest isolate with 4 cases(50%) followed by *T.mentagrophytes* 3 (37.5%) and *M.gypseum* 1(12.5%). In tinea cruris out of the 5 culture isolates, T rubrum was the commonest isolate with 4 cases (80%) followed by T mentagrophytes 1 (20%) .In tinea unguium out of 5 culture isolates, *T.rubrum* was the commonest isolate with 3 cases (60%) followed by *T.mentagrophytes* 2 (40%). In tinea capitis ,all 2 culture isolates were *T.tonsurans* (100%). In tinea pedis ,*T mentagrophytes* 1(100%) was isolated. In tinea faciei, *T rubrum* 1(100%) was isolated. Overall out of 22 culture isolates, *T.rubrum* was the most common isolate with 12 cases (54.54%), followed by *T.mentagrophytes* 7 (31.81%), *T.tonsurans* 2 (9%) and *M.gypseum* 1(4.5%). (**Table-5)**

Table-5 : Dermatophytes isolated in relation to clinical types

Clinical type	No.	*T. rubrum*	*T. mentagrophyte*	*T.tonsurans*	*M.gypseum*	Total isolated
Tinea corporis	35	4(50%)	3(37.5%)	-	1(12.5%)	8
Tinea cruris	17	4(80%)	1(20%)	-	-	5
Tinea unguium	12	3(60%)	2(40%)	-	-	5
Tinea capitis	6	-	-	2(100%)	-	2
Tinea pedis	2	-	1(100%)	-	-	1
Tinea faciei	2	1(100%)				1
Tinea manuum	1	-	-	-	-	0
Total	75	12	7	2	1	22

DISCUSSION

The present study included 75 clinically diagnosed cases of dermatophytosis patients attending the Dermatology and Venereology outpatient department of IMS & SUM HOSPITAL, Bhubaneswar.

In this study, dermatophytosis was more common in the age group of 31-40 years (24 %) followed by 21-30 years (21.33 %), which is comparable with other studies done by Veer P et al [9], Jain N et al [10], whereas Jesudanam TM et al [11], Sen SS et al [4] reported that the most common age group affected was 21-30 years. In the present study, males (72%) were more commonly affected than females (28%). Male to female ratio was 2.57:1, which is comparable with previous studies by Siddappa K et al [12], Karmakar S et al [13], Huda MM et al [14], Bindu V et al [15], Sumana V et al [5] and Sen SS et al [4], whereas Bhokari MA et al [16] and Jesudanam TM et al [11] reported that females were commonly affected than males, with male to female ratio being 1:2.6 and 1:1.08 respectively.

Male predominance could be due to increased outdoor physical activities and increased opportunity for exposure to infection than females.

In the present study, tinea corporis was the commonest clinical type encountered (46.66%) followed by tinea cruris (22.66%) and the commonest age group affected was 21 -30 years (22.85%). Males (68.57%) were predominantly affected than females (31.42 %). These findings are comparable with other studies done by Bindu V et al [15] (54.6%), and Sen SS et al [4](48%).

In the present study, tinea cruris was the second commonest clinical type (22.66%) and the commonest age group affected was 21-30 years and 31-40 years each with 4 cases (23.52%). Males (82.35%) were more commonly affected. The above findings are comparable with previous studies done by Siddappa K et al [12], Bhokari MA et al [16], and Sen SS et al [4].

In the present study, onychomycosis was more common in males. Male to female ratio was 2:1, which is comparable with other studies done by Singh S et al [17] and Vijaya D et al [18], whereas Bhokari MA et al [16] and Jesudanam TM et al [11] in their study reported that females were commonly affected than males, with male to female ratio being 1:2.6 and 1:1.08 respectively.

In the present study, tinea capitis was more commonly seen in the age group of 0-10 years

(50%), which is comparable with other studies done by Siddappa K et al [12](77.78%), Reddy BNS et al [19](73.5%) and Karmakar S et al [13] (85.5%).

High occurrence of tinea capitis in less than 10 years of age may be due to lack of fungistatic sebum by the scalp in childhood. Female preponderance of tinea capitis reported by several workers may be due to hormonal changes, closeness to children, and more visits to the hairdresser, whereas the reported lower incidence in females may be due to the custom of regular application of vegetable oil over the scalp which has fungistatic properties.

In the present study, out of 75 cases, tinea pedis was seen in 2.6% of cases, which is comparable with the study done by Karmakar S et al [13] (2%) and Bindu V et al [15] (3.3%), whereas Huda MM et al [14] and Singh S et al [3] in their study on dermatophytosis, reported tinea pedis in 7% and 11.53% cases respectively. In the present study, tinea faciei was seen in 2.6% of cases, which is comparable with other studies done by Huda MM et al [14](1% cases) and Singh S et al [3] (1.58% cases) whereas Karmakar S et al [13] have reported tinea faciei in 6% cases.

In the present study, out of 75 cases of dermatophytosis, tinea manuum was seen in one case (1.33%), which is comparable with other studies done by Siddappa K et al [12] **(**1.53%) and Huda MM et al [14] (3%).

In the present study, infection was most common in the low-income group 50.66% followed by the middle-income group 32 % and the high-income group 17.33%, which is similar to the observation of Ranganathan S et al [8] who reported that 69.2% of infected people were from low-income group and 23.2% from middle-income group. This may be due to poor hygienic conditions, overcrowding, sharing clothes without washing them properly, and also due to poor nutrition.

In the present study, dermatophytosis was most commonly seen in manual workers 40 (53.33%), which included agricultural workers and manual labourer, followed by household workers (mainly housewives) 19 (25.33%), students 12 (16%) and other miscellaneous 4 (5.33%) which included professionals, service and business class workers. The above findings are comparable with the observations of Veer P et al [9] and Sumana V et al [5]. This could be due to increased physical activity and opportunity for exposure in the case of manual workers and increased wet work in the case of housewives.

In the present study, out of 75 clinically diagnosed cases of dermatophytosis, 52 cases (69.34%) were positive for fungi, either by KOH and/or culture. Twenty cases (38.46%) were positive by both KOH and culture, 30 cases (57.6%) were positive by KOH and negative by culture, 2 cases (2.67%) were negative by KOH but culture positive. These findings are comparable with other studies done by Sumana V et al [5], Karmakar S et al [13]**,** Singh S et al [3]**,** and Bindu V et al [15]**.** This variation could be due to the non-viability of fungal elements in some cases.

In the present study, *T. rubrum* 12 (54.54%) was the commonest aetiological agent in the majority of clinical types followed by *T.mentagrophytes* 7 (31.81%), *T.tonsurans* (9%), *M.gypseum* (4.5%) which is comparable to other studies done by Bindu V et al [15], Ranganathan S et al [8], Singh S et al [17] and Jain N et al [10]**.** In tinea capitis, *T.tonsurans* was isolated in 2 cases (9%), which is comparable to other studies done by Bindu V et al [15], whereas Karmakar S et al [13] in his study, reported *T.violaceum* as the predominant isolate.

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CONCLUSION

Dermatophyte infections are highly prevalent in regions with hot and humid climates, particularly in areas with poor hygiene. The infection predominantly affects individuals between 31-40 years of age, with a higher incidence in males, especially those engaged in manual labourer or household work. The trunk and groin are the most commonly affected areas, leading to frequent cases of tinea corporis and tinea cruris. Laboratory identification through microscopy and culture confirms that *T. rubrum* is the most common species involved, followed by *T. mentagrophytes* and *T. tonsurans*. This highlights the need for targeted preventive measures and effective treatment strategies in these high-risk groups.

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