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Original Research Article

Prevalence of dermatophytic infections including antifungal susceptibility pattern of dermatophytes in a tertiary care hospital

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ABSTRACT

Background: Dermatophytosis is a commonly encountered superficial fungal infection in the tropical and subtropical countries. The present study was undertaken to study the clinicomycological profile of dermatophytosis and perform antifungal susceptibility testing for the isolated dermatophytes.
Methods: This is 2 years cross- sectional observational study including 433 clinically suspected cases of dermatophytoses. Skin, hair and nails were collected, subjected to direct microscopy by Potassium hydroxide (KOH) mount and culture on Sabouraud's Dextrose Agar (SDA) with chloramphenicol and cycloheximide. Positive growth on culture media was further identified by LPCB mount, slide culture test, growth on Dermatophyte Test Medium, pigment production in corn meal agar with 1% dextrose, urease test and hair perforation test. The isolated dermatophytes were subjected to antifungal susceptibility testing by agar based disc diffusion method.
Results: Out of 433 samples, fungal filaments were seen in 308 (71.1%) samples by KOH mount, 259 (59.8%) dermatophytes were isolated from culture. Males (60.5%) were more commonly affected than females (39.5%). Most common age group affected was 21-30 years. Trichophyton mentagrophytes (57.5%) was the most common isolate followed by Trichophyton rubrum (30.1%). Tinea corporis was the most common clinical presentation (52.7%) followed by Tinea unguium (14.1%). Antifungal susceptibility testing showed itraconazole as the most sensitive antifungal agent, while fluconazole was least sensitive.
Conclusions: This study provides a scope for assessment of prevalence and clinicomycological profile, which could help in estimation of the problem and hence prevent spread of dermatophytoses with adequate control measures.

Keywords: Dermatophytosis, Dermatophytes, Tinea, *Trichophyton*

INTRODUCTION

Superficial fungal infections are among one of the most frequent forms of human infection, being estimated to affect about 20-25% of the global population.[1] Dermatophytosis is a common superficial fungal infection in our country. The prevalence of dermatophyte infection varies from place to place, depending on the geographical location and climatic conditions. Other factors like poverty, poor hygiene, immune status and occupation also play important role in the development of the disease.[2] Although considered as a trivial disease, physiological effects of dermatophytoses are quite high, possibly due to stigma attached, cosmetic involvement, chronicity and being costly to treat.

Dermatophyte infections ('Ring worm' or 'Tinea') are caused by a group of keratinophilic fungi known as

dermatophytes (meaning 'skin plant'), which invade dead keratinised layers of skin, hair and nail. They include three genera: *Trichophyton*, *Microsporum* and *Epidermophyton*.[3] Based on host preference and natural habitat, dermatophytes are grouped into three categories: Anthropophilic, Zoophilic and Geophilic. The clinical manifestations vary depending on the site affected and strain of dermatophyte.[4] Despite being a common disease, dermatophyte infections can be difficult to identify, possibly due to misuse of 'over-the-counter' topical corticosteroids, which can mask the actual appearance of the lesions. Other diseases resembling dermatophyte lesions need to be ruled out for proper diagnosis and management.[5] Odisha, in particular Bhubaneswar, has a warm and humid climate. Therefore, the population of this city is quite vulnerable to dermatophyte infections. The present study was undertaken to find out the prevalence of various clinical types of dermatophytoses, establish a correlation between clinical signs, symptoms and mycological findings and performing antifungal susceptibility testing of isolated dermatophytes.

METHODS

This is a cross- sectional observational study done over a period of 2 years from November 2016 to October 2018. The study population comprised of 433 clinically suspected cases of dermatophytoses attending the Dermatology Out Patient Department of Hi-Tech Medical College and Hospital, Bhubaneswar. Patients on antifungal drug therapy (topical/ systemic) or have taken antifungal drugs in the last 3 months were excluded from the study. Ethical clearance for the study was obtained from the institutional ethics committee before commencement of the study

Collection and processing of samples

After taking informed consent, patient's details (name, age, sex, occupation, site of lesion, duration of illness, signs and symptoms, any other illness, medications) were recorded. Clinical samples in the form of skin, hair and nails were collected and processed as per standard mycological procedures. Affected areas were cleaned with 70% alcohol and allowed to dry. Skin was scraped from active margins of lesions with the help of a sterile blunt scalpel, nails were collected by clipping and hairs were plucked with sterile forceps, collected in sterile brown paper packets and labelled. One part of the sample was directly observed under microscope by potassium hydroxide (KOH) mount. Another part of the sample was inoculated on Sabouraud's Dextrose Agar (SDA) with chloramphenicol (0.05mg/ml) and cycloheximide (0.5mg/ml).

Direct microscopic examination

A small quantity of sample (skin, hair) was placed in a drop of 10% KOH solution on a clean glass slide, preparation was kept aside for 30 minutes, then observed microscopically (400X magnification) for presence of fungal elements (Figure 1).

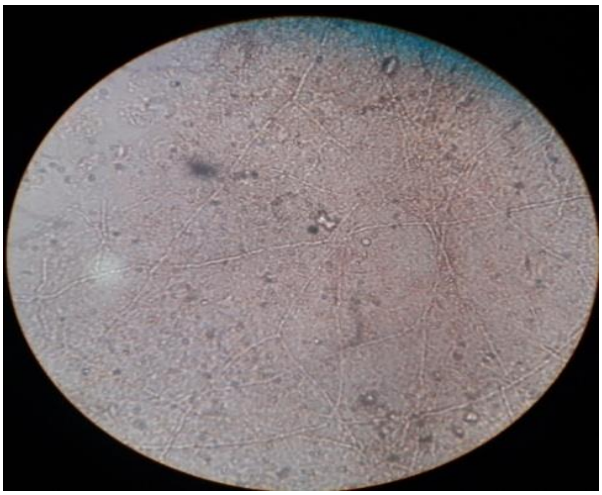

Figure 1: Fungal elements under KOH mount (400 X).

Nail samples were placed in 20% KOH solution with 40% Dimethyl sulfoxide (DMSO) and observed. In case of very thick nail specimens, preparation was kept in a moist chamber and observed next day.

Culture

The samples (skin, hair, nails) were cut into small pieces approximately 1-2mm in size and inoculated on SDA slants with chloramphenicol and cycloheximide irrespective of their KOH positivity and incubated at 25 °C in a BOD (Biological Oxygen Demand) incubator for 4 weeks.

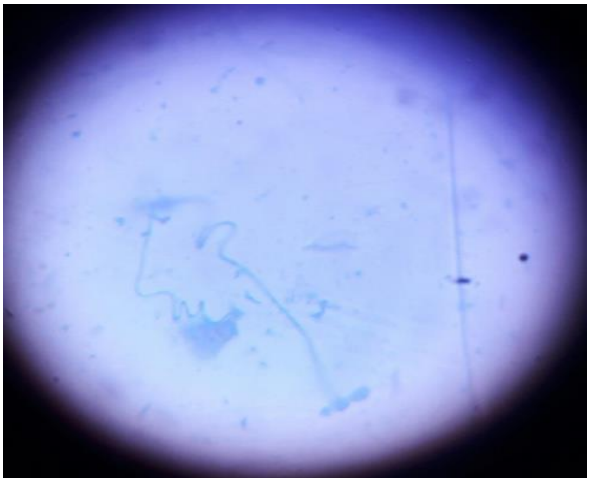

Figure 2: Spiral hyphae of *Trichophyton mentagrophytes* under LPCB mount.

Tubes were observed for growth at least twice during the first week, and once a week thereafter, for a total of 4 weeks. Colony morphology, rate of growth, pigment production on obverse and reverse in the test tubes were noted. If there was no growth in the test tubes after four

weeks, it was taken as negative for fungal growth. The growths obtained were examined microscopically by LPCB (Lactophenol cotton blue) mount. Size, shape, arrangement of microconidia and macroconidia, type of hyphae, any special structures like favic chandelier, spiral hyphae etc. were noted, in order to identify the dermatophytes (Figure 2).

Figure 3: *Trichophyton mentagrophytes* on Dermatophyte test media.

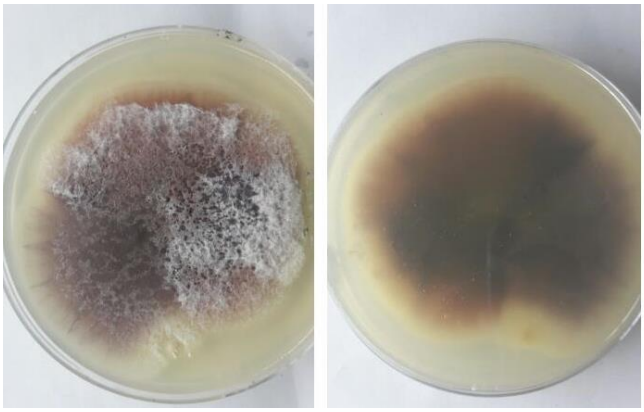

Figure 4: *Trichophyton rubrum* on Corn meal agar with 1% dextrose.

Other tests like slide culture technique, growth on special media like Dermatophyte Test Media (DTM) and Corn Meal Agar (CMA) with 1% dextrose, Hair Perforation Test and biochemical tests like Urease test were performed when necessary.

All media were procured from Hi-Media, Mumbai and prepared according to manufacturer's instructions. The fungal colonies on SDA, which were confirmed as Dermatophytes, were inoculated on DTM plates and slants, incubated at 25 °C in BOD incubator and observed for colour change. The dermatophytes produce red colour in the medium (Figure 3).

On corn meal agar medium with 1% dextrose, *Trichophyton rubrum* produces a deep red pigment (Figure 4). In order to differentiate between *Trichophyton rubrum* and *Trichophyton mentagrophytes*, two tests were performed: urease test and hair perforation test.

Urease test

Christensen's urease agar slants were inoculated and kept in BOD incubator at 30 °C. *Trichophyton mentagrophytes* (urease positive), produced pink colour within a few days, while *Trichophyton rubrum* showed no colour change.

In -vitro hair perforation test

Hair perforation test was performed to differentiate between *Trichophyton mentagrophytes* (positive) and *Trichophyton rubrum* (negative). Presence of wedge shaped perforations on hair indicated a positive test.

Antifungal susceptibility testing

Recently CLSI (Clinical and Laboratory Standards Institute) has approved a micro broth dilution method for antifungal susceptibility testing of molds, but these tests are cumbersome and difficult to be performed in routine laboratory set up. The agar based disc diffusion (ABDD) is an easy method to determine the antifungal susceptibility of dermatophytes, but data regarding these methods are scarce and not standardised.[6,7] The application of *in vitro* antifungal susceptibility testing for guidance of antifungal drug therapy has been limited due to uncertain correlation between *in vitro* and *in vivo* action of drugs.[8,9] Antifungal susceptibility testing of the isolated dermatophytes was performed by agar based disc diffusion method for 5 antifungal drugs.[6,7] The antifungal discs were procured in readymade form from Hi-Media (Mumbai, India). They are as follows: Ketoconazole (KT): 10µg/disc, Fluconazole (FLC): 10µg / disc, Clotrimazole (CC): 10µg/disc, Itraconazole (IT): 10µg/ disc, Nystatin (NS): 100U/ disc. The isolates were subcultured on Potato Dextrose Agar (PDA) at 28 °C for 7 days to enhance sporulation. The growth was harvested in sterile saline and the suspension was adjusted to 1 x 10^6 /ml using a hemocytometer. Plates of Mueller Hinton Agar (MHA) of 4mm depth were inoculated. The surface of the MHA plate was streaked in 4 different directions (90°) to cover the entire surface, then kept aside for a few minutes for drying and discs were applied using sterile forceps. *Trichophyton mentagrophytes* ATCC 9533 and *Trichophyton rubrum* ATCC 28188 strains served as control. The plates were incubated in a BOD incubator at 25°C for 5-10 days. After sufficient growth occurred, the diameters of zones of inhibition surrounding the antifungal discs were measured and results interpreted.[6,7,10]

RESULTS

Out of 433 clinically suspected cases of dermatophytoses, males (60.5%) were more affected than females (39.5%) with male: female ratio 1.5:1. Most of the affected patients belonged to the age group of 21-30 years (25.4%) followed by 31-40 years (20.3%) (Table 1).

Table 1: Distribution of patients according to age and sex (n=433).

Age group (in years)	Males	Females	Total
1-10	15 (3.5%)	11 (2.5%)	26 (6%)
11-20	43 (9.9%)	30 (6.9%)	73 (16.8%)
21-30	59(13.6%)	51 (11.8%)	110 (25.4%)
31-40	56(12.9%)	32 (7.4%)	88 (20.3%)
41-50	40 (9.2%)	19 (4.4%)	59 (13.6%)
51-60	27 (6.2%)	20 (4.6%)	47 (10.8%)
>60	22 (5.2%)	8 (1.9%)	30 (7.1%)
Total	n_1=262 (60.5%)	n_2=171 (39.5%)	n=433 (100%)

Table 2: Distribution of clinical types of dermatophytoses (n=433).

Clinical types	Males	Females	Total
Tinea corporis	145 (33.5%)	83 (19.1%)	228 (52.6 %)
Tinea unguium	33 (7.6%)	28 (6.5%)	61 (14.1 %)
Tinea cruris	30 (6.9%)	22 (5.1%)	52 (12 %)
Tinea faciei	16 (3.7%)	14 (3.2%)	30 (6.9 %)
Tinea pedis	13 (3%)	7 (1.6%)	20 (4.6 %)
Tinea capitis	9 (2.1%)	10 (2.3%)	19 (4.4 %)
Tinea manuum	10 (2.3%)	5 (1.2%)	15 (3.5 %)
Tinea incognito	3 (0.7%)	2 (0.5%)	5 (1.2 %)
Tinea barbae	3 (0.7%)	-	3 (0.7 %)
Total	262 (60.5%)	171 (39.5%)	433 (100 %)

A chi-square test was fitted to the above table where value of chi-square (χ^2) = 7.3 with 6 degrees of freedom.

The test was found not statistically significant with p-value >0.5. This indicates that prevalence of dermatophytoses in males and females are not significantly different. Majority of the affected patients belonged to low socioeconomic status and were involved in active physical work, like manual labourers, farmers, domestic help etc. Tinea corporis (52.7%) was the most common clinical presentation, followed by Tinea unguium (14.1%) and Tinea cruris (12%) (Table 2). A chi-square test was applied to the above table after clubbing three clinical types like Tinea manuum, Tinea barbae and Tinea incognito to get rid of expected frequency of any cell being >5. The value of chi-square (χ^2) = 5.03, degree of freedom =6, p-value >0.5 at 5% level of significance and the test was found to be not statistically significant. It indicates that there is no difference between males and females with relation to clinical presentations.

Table 3: Correlation between results obtained by direct microscopy (KOH mount) and culture (n=433).

KOH results	Number of cases		
	Culture (+ve)	Culture (-ve)	Total
KOH (+ve)	251 (57.9%)	57 (13.2%)	308 (71.1%)
KOH (-ve)	8 (1.9%)	117 (27%)	125 (28.9%)
Total	259 (59.8%)	174 (40.2%)	433 (100 %)

Table 4: Correlation between clinical presentations and isolated dermatophytes.

Clinical types	T. mentagrophytes	T. rubrum	T. tonsurans	M. nanum	Total
Tinea corporis	65	36	16	8	125
Tinea unguium	28	11	-	-	39
Tinea cruris	26	15	3	3	47
Tinea faciei	11	6	-	-	17
Tinea pedis	7	3	-	-	10
Tinea capitis	5	2	2	-	9
Tinea manuum	4	1	-	-	5
Tinea incognito	2	3	-	-	5
Tinea barbae	1	1	-	-	2
Total	n_1=149 (57.5%)	n_2=78 (30.1%)	n_3=21 (8.1%)	n_4=11 (4.3%)	n=259 (100%)

Out of 433 samples processed, 308 (71.1%) were positive for KOH mount while 259 (59.8%) were culture positive. Out of 308 KOH positive samples, 251 (57.9%) were both KOH positive and culture positive, rest were culture negative (Table 3). A chi-square test was fitted to this 2 x 2 contingency table and chi square (χ^2) = 208.49, with 1 degree of freedom. This proves that the test is highly statistically significant at 95% confidence interval with p-value <0.0001.

Out of 259 isolated dermatophytes, *Trichophyton mentagrophytes* (57.5%) was the most common isolate followed by *Trichophyton rubrum* (30.1%), *Trichophyton tonsurans* (8.1%), and *Microsporum nanum* (4.3%). None

of the *Epidermophyton* species were recovered. All four isolated dermatophyte species were recovered from Tinea corporis, the most common clinical presentation (Table 4). Antifungal susceptibility testing showed itraconazole as the most sensitive antifungal agent, while fluconazole was the least sensitive (Table 5).

Table 5: Antifungal susceptibility pattern of isolated dermatophytes.

Antifungal discs	S/R*	T. mentagro phytes (N=149)	T. rubrum (N=78)	T. tonsurans (N=21)	M. nanum (N=11)
Fluconazole	S	4 (2.7%)	4 (5.2)	1 (4.8%)	1 (9.1%)
	R	145 (97.3%)	74 (94.8%)	20 (95.2%)	10(90.9%)
Itraconazole	S	146 (97.9%)	74 (94.8%)	18 (85.7%)	9 (81.8%)
	R	3 (2.1%)	4 (5.2%)	3 (14.3%)	2 (18.2%)
Ketoconazole	S	38 (25.5%)	19 (24.4%)	7 (33.3%)	3 (27.2%)
	R	111 (74.5%)	59 (75.6%)	14 (66.7%)	8 (72.8%)
Clotrimazole	S	144 (96.7%)	57 (73.1%)	15 (71.4%)	10(90.9%)
	R	5 (3.3%)	21(26.9%)	6 (28.6%)	1 (9.1%)
Nystatin	S	143 (95.9%)	71(91.1%)	17 (80.9%)	9 (81.8%)
	R	6 (4.1%)	7 (8.9%)	4 (19.1%)	2 (18.2%)

*[S- Sensitive, R- Resistant]

DISCUSSION

Out of 433 patients, 262 (60.5%) were males and 171 (39.5%) were females, with male: female ratio being 1.5:1. Similar findings with male predominance were seen in studies conducted by Sudha M et al, showed 62.3% males and 37.7% females (1.65:1), Gunasekaran P, showed 62.3% males and 37.7% females (1.5:1).[11,12] Maximum cases of dermatophytoses were seen in the age group of 21-30 years (25.4%) followed by age group of 31-40 years (20.3%). In the present study, there is low occurrence of the disease in both extremes of life, i.e. in young children (1-10 years) and persons above 60 years (6% and 7.1% respectively). Similar findings have been noted in studies done by Walke HR et al.[13] Majority of the affected patients were males in 2nd and 3rd decades of life, engaged in active physical labour like manual labourers, farmers, domestic help etc. and belonged to low socioeconomic status. High occurrence of dermatophytoses in such patients might be due to sweating from strenuous outdoor physical activity, exposure to infected animals, soil, poor personal hygiene, lack of awareness about the disease etc. Studies by Ghosh RR et al, and Sudha M et al, also conform to these findings.[11,14] Lower incidence in females could be attributed to their ignorance to seek medical advice, especially in patients from rural areas.

Tinea corporis (52.65%) was the most common clinical presentation followed by Tinea unguium (14.1%) and Tinea cruris (12%). This is in accordance with the findings of Monika K et al, where the most common clinical presentation was Tinea corporis (31%) followed by Tinea unguium (21%).[15] High rates of Tinea corporis could be attributed to its symptomatic nature (pruritus) which leads the patient to seek medical advice.[13] Studies by Walke HR et al, Sudha M, showed Tinea corporis as the most common clinical type followed by Tinea cruris which stands against present study.[11,13] However, studies by Gupta CM et al, and Ghosh RR et al, showed Tinea unguium as the most common clinical presentation followed by Tinea corporis and Tinea capitis.[5,14] The other clinical presentations of dermatophytoses observed in our study were Tinea faciei (6.9%), Tinea pedis (4.6%), Tinea capitis (4.4%), Tinea manuum (3.5%), Tinea incognito (1.1%) and Tinea barbae (0.7%). Almost similar findings were observed in studies done by Roopa C et al, Dhyaneswari GP et al.[16,17]

In the present study, out of 433 clinical samples, 308 (71.1 %) samples were positive by direct microscopy by KOH mount and 259 (59.8%) samples were culture positive. Out of 308 KOH positive samples, 251 (57.9%) samples were both KOH positive and culture positive, while the rest 57 (13.2%) were culture negative. The direct microscopy and culture findings of present study are relatively in agreement with study done by Dhyaneswari GP et al, (72.6% KOH positive) and Mahale RP et al, (61.01% culture positive).[17,18] There is a difference between KOH positivity rate and culture positivity rate i.e. fungal elements were seen under direct microscopy but samples failed to grow on culture which might be due to various factors like usage of topical corticosteroids, unsatisfactory collection of samples containing dead fungal hyphae.[19,20]

Authors also came across instances where no fungal elements were seen under direct microscopy but showed growth on culture. This might be due to presence of scanty fungal elements which were missed during direct microscopic examination or due to presence of fungal

elements in inactive sporulating form, which could not be visualised under direct microscopy.[16,20]

Trichophyton mentagrophytes (57.5%) was the most commonly isolated dermatophyte in our study, followed by *Trichophyton rubrum* (30.1%), *Trichophyton tonsurans* (8.1%) and *Microsporum nanum* (4.3%). Present study correlates with studies done by Gadadavar S et al, (*Trichophyton mentagrophytes* 81.8%, *Trichophyton rubrum* 11.36%) and Bhatia VK et al, (*Trichophyton mentagrophytes* 63.5%, *Trichophyton rubrum* 35.1%).[18,21] However, other studies show *Trichophyton rubrum* as the most common isolate followed by *Trichophyton mentagrophytes*,which are as follows: Dhyaneswari GP et al, (*Trichophyton rubrum* 59.6%, *Trichophyton mentagrophytes* 26%), Walke HR et al, (*Trichophyton rubrum* 56.37%, *Trichophyton mentagrophytes* 19.39%).[13,17] In present study none of the *Epidermophyton spp*. were isolated which was also observed in studies done by Bhatia VK et al, and Gadadavar S et al.[3,21] Out of 125 isolates obtained from Tinea corporis, 65 isolates were *Trichophyton mentagrophytes*, 36 were *Trichophyton rubrum*, 16 *Trichophyton tonsurans* and 8 isolates were of *Microsporum nanum*.

Most clinical types of dermatophytoses respond well to topical antifungal therapy, while Tinea unguium, Tinea capitis and extensive type of dermatophytoses require systemic therapy. Recently, there has been a rise in antifungal resistant strains of fungi. Therefore, early initiation of correct antifungal therapy is essential for proper treatment and prevention of spread of disease. In the present study, antifungal susceptibility testing by agar based disc diffusion method [6,7] was performed for five antifungal drugs: ketoconazole, fluconazole, itraconazole, clotrimazole and nystatin. Itraconazole (97.9%) was the most sensitive followed by clotrimazole (96.7%). fluconazole (2.7%) was the least sensitive. Present study findings are almost similar with the findings of Pakshir K et al, (97.5%) was the most sensitive antifungal drug while fluconazole (2.5%) was least sensitive.[10]

CONCLUSION

Dermatophytosis is a common yet important superficial fungal infection affecting people of all age groups. The disease is quite challenging to treat, particularly when associated with immunocompromised states like HIV infection, diabetes mellitus etc., which can increase the severity of infection. Direct examination of samples by KOH mount is a quick, simple and inexpensive method of diagnosis of dermatophytosis, but there are chances of technical errors in diagnosis. Definitive diagnosis can be made on the basis of growth in culture, but it has the disadvantage of being time consuming. Therefore, the diagnosis of dermatophytoses should be confirmed by simultaneous examination of KOH mount as well as culture. The treatment of the disease would be more effective and meaningful when antifungal agents are prescribed based on culture and antifungal susceptibility results. This study provides an insight into the various clinical presentations, etiological agents and their antifungal susceptibility pattern in this region which could help in evaluation of the problem for efficient screening and treatment of the dermatophytic infection.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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