




Species Distribution and Antifungal Susceptibility Profile of Dermatophytes from a Tertiary Care Centre in North India

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Abstract

Objective Dermatophytoses, one of the most ancient diseases, is becoming a menace in recent times. This has made the knowledge of antifungal susceptibility a priority in today's times.

Material and Methods This is a prospective study conducted over 18 months including all dermatophytes isolated during the period. Dermatophytes were identified by routine phenotypic methods. Antifungal susceptibility testing was performed for griseofulvin, terbinafine, and itraconazole as per the Clinical Laboratory Standard Institute M38 A2, and minimum inhibitory concentrations (MICs) were read after 5 days.

Results Patient details and associated risk factors were recorded. Fixed dose combinations with steroids were associated with 79.3% (46 out of 58) of patients with dermatophytosis of skin. Among the 72 dermatophytes isolated during the study period, 58 (80.5%) were isolated from skin scrapings and 14 (19.4%) from nail samples. Tinea corporis with cruris was the most common presentation. The most common dermatophyte isolated from skin scrapings was *Trichophyton mentagrophytes* complex (70.6%, 41 out of 58), while from nail samples it was *Trichophyton rubrum* complex (78.57%, 11 out of 14). Based on the MIC₅₀ and MIC₉₀ results, itraconazole showed the lowest MICs, followed by terbinafine and then griseofulvin.

Conclusion With the changing epidemiology of species distribution and antifungal resistance, there is a need for continuous surveillance of these parameters of dermatophytes.

Keywords

- ▶ Dermatophytes
- ▶ *Trichophyton*
- ▶ antifungal susceptibility
- ▶ Griseofulvin
- ▶ Terbinafine

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Introduction

Dermatophytosis in India, especially in the last 4 to 5 years, has become a challenging public health problem with the given rising trend.^{1,2} The rise in chronic and recurrent incidents and re-infection in susceptible populations and treatment failure are a concern.³ The factors that have contributed to this are poor compliance with therapy, steroid use, self-medication, and possible antifungal resistance.³ *Trichophyton rubrum* has been the commonest dermatophyte for ages, but now the trend has been changing in some parts of the world.⁴

The antimicrobial drug resistance is an inevitable part of the process of evolution in the microbial world. Earliest resistance in dermatophytes was seen in griseofulvin.⁵ The recent studies have shown that some of the patients are not responding to the routinely prescribed drugs like griseofulvin and terbinafine due to resistance and the patients are taking drugs for longer period of time with no response.⁶ Clinically, antifungal drug resistance may be suspected in patients who do not respond to the first-line therapy, generalized or atypical presentation, and recurrent episodes of infection.⁵ It has become a challenging task now to treat patients with chronic dermatophytosis.⁶ The present study was undertaken to study the species distribution and antifungal profile of various dermatophytes isolated in patients from our setting.

Material and Methods

This was a prospective observational study conducted in the Department of Microbiology in collaboration with the Department of Dermatology, Venereology and Leprosy at Government Medical College Hospital, Chandigarh, India. The study was conducted over a period of 18 months from January, 2018 to June, 2019. A total of 72 samples (58 skin scraping and 14 nail samples) which were potassium hydroxide (KOH) mount positive and fungal culture positive for dermatophytes were included in the study. No hair sample was received during this period. A detailed case history, examination, and other relevant workup were done and noted down on a proforma after taking an informed consent from the patient.

Clinical specimens were subjected to slide or tube KOH and examined for thin, septate branched hyphae, or arthroconidia in chains. Samples were inoculated on Sabouraud dextrose agar (SDA, HiMedia, India) tube slants with chloramphenicol, gentamicin, and with and without cycloheximide. Each medium was inoculated in duplicate, incubated at 25°C and 37°C, and observed for fungal growth daily for 1 week and twice weekly for the next 3 weeks. The tube that grew mold was identified on the basis of morphological features like growth rate, texture, and color of the colony on obverse and reverse of SDA, microscopic examination by lactophenol cotton blue mount and slide cultures, and identified by standard mycological methods.⁷ Antifungal susceptibility testing (AFST) was performed by the micro-broth dilution technique for griseofulvin, terbinafine, and itraconazole as per the Clinical Laboratory Standard Institute

(CLSI) "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi" (M38 A2).⁸ *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality strains. The tested antifungals were commercially sourced as powders from Sigma-Aldrich, China. Minimum inhibitory concentrations (MICs) were read after 5 days of incubation at 35°C. MIC for drugs griseofulvin, terbinafine, and itraconazole was defined as the lowest concentration of antimicrobial agent that caused a reduction of 80% or greater growth in comparison to growth control (drug-free media with inoculum).⁸ The study conducted was approved by the ethical committee of the institute.

Results

During the study period, a total of 189 skin scrapings and 306 nail samples were received. No hair samples were received. Skin scrapings showed the positivity of 88.9% (168 out of 189) on KOH microscopy, while it was 58.2% (178 out of 306) for nail samples. One hundred and sixty-nine skin scrapings out of 189 were put up for culture due to insufficient samples. Culture positivity for dermatophytes in skin scrapings was 52.1% (88 out of 169) and in nail samples 11.8% (36 out of 306). A total of 72 dermatophyte isolates from skin scrapings and nail samples were a part of the study. Out of these 72 isolates, 58 (80.5%) were isolated from skin scrapings and 14 (19.4%) from nail samples. Overall, the mean and median age of patients was 30.5 years for both. For skin scrapings, the mean age was 28.4 years and median 28.5 years, whereas for nail samples, the mean age was 39 years and median 37 years. The ratio of male to female was 5:3 for all the cases, 16:13 (55% male and 45% female) for skin scrapings and 13:1 (93% male and 7% female) for nail samples.

The most common clinical form of tinea in the 58 cases was tinea corporis with cruris which was present in 21 (36.2%) patients, followed by tinea corporis (19, 32.7%). Third most common presentation was tinea cruris which was seen in 11 (18.9%) patients. In five cases, face was involved in which two cases were each of tinea faciei and tinea faciei with corporis and one case of tinea faciei with corporis and cruris. There was one case of extensive tinea corporis with cruris which involved greater than 50% body. This case had diabetes mellitus and used fixed dose steroid combinations. Thirty-seven (63.8%) patients of dermatophytoses of skin gave a history of similar illness in other family members with 23 (39.6%) patients having two family member involvement. Maximum five-member transmission in family was seen in 2 out of 58 (3.4%) patients, whereas 21 out of 58 (36.2%) patients had no similar illness among family members. Lesions varied from 2 to over 10, two to five lesions were seen in 36 out of 58 (62.1%) cases, six to nine lesions in 16 out of 58 (27.6%) cases, greater than 10 lesions in 4 out of 58 (6.9%) cases, and two had greater than 50% face involvement.

Out of 58 patients with dermatophytosis of skin, 46 (79.3%) had a history of use of fixed dose combination containing steroid (clobetasol/betamethasone, miconazole/clotrimazole/tolnaftate, neomycin, and chlorhexidine). Among the 14 nail

samples, a total of nine (64.2%) patients gave the history of trauma: six (42.8%) reported trauma only, two (14.2%) trauma with diabetes mellitus, and one (7.1%) trauma with the use of steroids. One patient (7.1%) each had the history of diabetes mellitus and the use of steroid. Maximum patients (30, 41.6%) presented to dermatology outpatient department (OPD) after 6 months to 1 year of illness. The second most common duration of presentation was from 1 to 2 years of illness (26, 36.1%) followed by 2 to 6 months of illness (12, 16.6%). For skin scrapings, the maximum number of patients attended dermatology OPD within 6 months to 1 year of illness, 24 (41.3%). For nail samples, maximum patients attended the dermatology OPD within 1 to 2 years of illness (8, 57.1%) followed by 6 months to 1 year of illness (6, 42.8%).

► **Table 1** shows species distribution of dermatophytosis. Overall, the most common causative agent of tinea was *Trichophyton mentagrophytes* complex which was 61.1% (44 out of 72) followed by *T. rubrum* complex which was 31.9% (23 out of 72). For skin scrapings, the most common causative agent of dermatophytosis was *T. mentagrophytes* complex which was 70.6% (41 out of 58) and *T. rubrum* complex which was 78.57% (11 out of 14) for nail samples. AFST was done by the micro-broth dilution method according to the CLSI document M38-A2. The AFST of *T. mentagrophytes* complex for griseofulvin, terbinafine, and itraconazole showed the MIC range of 0.25 to 64, 0.0156 to 4, and 0.0312 to 0.125 µg/mL, respectively. AFST of *T. rubrum* complex for griseofulvin, terbinafine, and itraconazole showed the MIC range of 0.25 to 128, 0.0156 to 2, and 0.03125 to 16 µg/mL, respectively (► **Table 2**).

Discussion

Dermatophytes have become the sleeping giant in today's era. Known as one of the oldest fungal infections, it has always been ignored as it does not usually have any acute presentation. This study was conducted for a period of 18 months, and 72 dermatophyte isolates were included in this study. Fifty-eight (80.6%) isolates of dermatophytes were from skin scrapings and 14 (19.4%) from nail samples. Most studies on dermatophytes have included the ones isolated from cases of dermatophytosis of skin, while in this study a significant number were also isolated from tinea unguium cases.^{3,9-12} Ansari et al reported 6.9% of dermatophytes isolated from tinea unguium patients in their study.¹⁰ The age group commonly affected in this study was 21 to 30 years and 31 to 40 years both accounting for 20 patients each. The mean age was 28.4 years for dermatophytosis of skin and 39 years for tinea unguium patients. These findings correlate well with other studies where 21 to 30 years is the commonest age group affected^{10,12,13} and the mean age also varies from 30 to 33 years in many studies on dermatophytosis of skin.^{4,14,15} The males significantly outnumbered the female patients, 62% (45) against 38% (27) in this study. Most studies have shown the similar pattern with a male preponderance in their studies.^{3,9-11,16,17} The number of male patients with tinea unguium (93%, 13 out of 14) was drastically higher in our study. Basically, young males were the

Table 1 Species distribution of dermatophytes isolated from skin and nail samples

Isolates of dermatophytes	Total (dermatophytosis)	Percentage (%)	Skin scraping (dermatophytosis of skin)	Percentage (%)	Nail (tinea unguium)	Percentage (%)
Total	72		58		14	
<i>Trichophyton mentagrophytes</i> complex	44	61.11%	41	70.69%	3	21.43%
<i>Trichophyton rubrum</i> complex	23	31.94%	12	20.69%	11	78.57%
<i>Trichophyton</i> species	4	5.56%	4	6.90%	-	-
<i>Microsporium gypseum</i>	1	1.39%	1	1.72%	-	-

Table 2 MIC distribution of dermatophytes isolated from skin and nail samples

Isolates	MIC (µg/mL)	Griseofulvin			Terbinafine			Itraconazole		
		Total	Dermatophytosis of skin	Tinea unguium	Total	Dermatophytosis of skin	Tinea unguium	Total	Dermatophytosis of skin	Tinea unguium
<i>Trichophyton mentagrophytes</i> complex	Numbers	44	41	3	44	41	3	44	41	3
	MIC range	0.25–64	0.25–64	0.5–1	0.0156–4	0.0156–4	0.125–0.5	0.0312–0.125	0.0312–0.125	0.03125–0.0625
	MIC ₅₀	1	1	0.5	0.25	0.25	0.25	0.0312	0.0312	0.0625
	MIC ₉₀	4	4	0.5	4	4	0.5	0.0625	0.0625	0.0625
	GM	0.868	0.888	–	0.210	0.207	–	0.038	0.037	–
<i>Trichophyton rubrum</i> complex	Numbers	23	12	11	23	12	11	23	12	11
	MIC range	0.25–128	0.25–128	0.25–0.5	0.0156–2	0.0156–2	0.0156–0.125	0.03125–16	0.03125–16	0.0312
	MIC ₅₀	0.25	0.25	0.25	0.0156	0.03125	0.0156	0.03125	0.03125	0.03125
	MIC ₉₀	1	128	0.5	0.25	2	0.0156	0.03125	0.03125	0.03125
	GM	0.564	1.059	0.283	0.036	0.066	0.019	0.041	0.052	0.031
<i>Trichophyton</i> species	Numbers	4	4	0	4	4	0	4	4	0
	MIC range	0.5	0.5	–	0.0312–2	0.0312–2	–	0.03125–0.0625	0.03125–0.0625	–
	MIC ₅₀	0.5	0.05	–	0.0625	0.0625	–	0.03125	0.03125	–
	MIC ₉₀	0.5	0.5	–	2	2	–	0.0625	0.0625	–
	GM	0.5	0.5	–	0.177	0.177	–	0.037	0.037	–
<i>Microsporum gypseum</i>	Number	1	1	0	1	1	0	1	1	0
	MIC	0.25	0.25	–	0.0156	0.0156	–	0.0625	0.0625	–

Abbreviations: GM, geometric mean; MIC, minimum inhibitory concentration.

most common as this is the working population in the hot humid climate of our country, and being the earning member of the family, they seek treatment earlier.

Prolonged use of fixed dose combination creams is known to result in an increased prevalence of recalcitrant clinical variants which is further responsible for treatment failure.^{17,18} Self-medication and easy availability of these ointments over the counter have made the situation more difficult.¹⁹ In this study, 79.3% (46) patients gave a history of use of these fixed combinations in dermatophytosis of skin as against 32.3% by another study.³ A total of four (5.5%) patients gave the history of diabetes mellitus which was observed as 6.1% patients by same study.³ Most patients in this study (24, 41.3%) presented in the period of 6 months to 1 year from the onset of symptoms followed by 31.0% (18) presenting after 1 to 2 years. Rudramurthy et al showed a slightly different pattern with 38.9% of patients presenting after 1 month to 6 months of disease followed by 6 months to 1 year (16.9%).³

The maximum number of patients in this study presented with tinea corporis with cruris (36.2%, 21) followed by tinea corporis (32.7%, 19) and tinea cruris (18.9%, 11). Poojary et al and other studies have shown a similar pattern with the combination of tinea corporis and tinea cruris being the commonest presentation.^{1,4,11,19} Some studies have tinea corporis as the commonest presentation,^{3,12,13} while some have shown tinea cruris as the commonest.^{9,10} Lesser numbers have been reported for tinea faciei and tinea capitis in this study which is also similar with other studies.^{3,10-12} Two studies have reported 51.7 and 69.2% of localized single lesions as against 48.2 and 30.8% of multiple lesions,^{3,11} while interestingly none of the patients in this study had a localized single lesion. All the patients had more than one lesion with the maximum number of patients having two lesions (15, 25.8%). Dermatophytosis is a disease of the family. It can spread by contact and sharing of things so most people give a family history for the same.²⁰ Two or more than two family members were infected in 63.8% (37) of patients with the maximum number of two family members having the disease (23, 39.6%). Pathania et al and Sardana et al have also reported a similar figure of 72 and 60% patients giving a family history of dermatophytosis.^{21,22}

T. rubrum has been the most common isolated dermatophyte for decades,^{23,24} but the epidemiology is now shifting to *T. mentagrophytes* being the commonest in some parts.^{11,25,26} Indian studies show this trend, wherein the studies from 2014 to 2016 show *T. rubrum* as the commonest dermatophyte^{13,27} and in the later years it is *T. mentagrophytes* which is commonest.^{3,9,28,29} In this study, *T. mentagrophytes* complex was the commonest isolate, followed by *T. rubrum* complex and one *Microsporum gypseum*. This is in complete sync with other studies from nearby areas.^{3,29} Studies from Iran have also reported *T. mentagrophytes* as the commonest dermatophyte,^{10,30} while studies from Turkey, Vietnam, Tunisia, Brazil, and Canada have reported *T. rubrum* as the commonest.^{14,31-35} *T. rubrum* still remains the commonest dermatophyte in most regions of the world even though the epidemiology is changing in some parts including ours. Sharma et al have reported a

significant number of *Trichophyton schoenleinii* (33.3%), being the second commonest dermatophyte in their study after *T. mentagrophytes*, a finding different from other studies.¹⁷ We could not isolate any *T. schoenleinii* in this study. Commonest dermatophyte in patients with tinea unguium was in contrast to skin dermatophytosis, with *T. rubrum* complex being the commonest dermatophyte (11 out of 14, 78.5%) as against only three (21.4%) *T. mentagrophytes* complex. The study by Kulkarni et al on tinea unguium also show *T. rubrum* as the commonest dermatophyte.³⁶ A study by Bitew having 54.6% of patients of tinea capitis showed a high number of *Trichophyton violaceum* (38.3%).³⁷ This implies that it is important to understand the epidemiology of the species distribution of dermatophytes in terms of the site of dermatophytoses.

AFST was performed for three drugs in this study griseofulvin, terbinafine, and itraconazole by the microbroth dilution method. Readings were taken after 5 days of incubation at 35° C. There is a huge variation in the incubating temperature and time of incubation for different studies. Most studies have incubated at 35°C, but the time period is not consistent.^{11,28,32,36,38} CLSI M38-A2 recommends incubating at 35°C for over 4 days when the growth control well shows sufficient (confluent) growth.⁸ Itraconazole in this study gave the lowest MICs for both *T. mentagrophytes* complex and *T. rubrum* complex, MIC₉₀ of 0.0625 µg/mL and 0.03125 µg/mL, respectively. Griseofulvin and terbinafine both gave an MIC₉₀ of 4 µg/mL for *T. mentagrophytes* complex, while MIC₅₀ for *T. rubrum* complex was higher with griseofulvin. In concise, based on the MIC₅₀ and MIC₉₀ results itraconazole showed the lowest MICs followed by terbinafine and then griseofulvin. Same trends have been reported by most authors,^{3,10,21,29,30} while Singh et al have reported higher MIC₉₀ for terbinafine than griseofulvin²⁸ and Salehi et al have shown higher MIC₉₀ of itraconazole than terbinafine.¹²

Griseofulvin showed a high MIC₉₀ of 4 µg/mL in this study for *T. mentagrophytes* complex and 1 µg/mL for *T. rubrum* complex. Few studies report similar values,^{10,28} while very high MIC₉₀ of 64 and 128 µg/mL have been reported by Rudramurthy et al and Pathania et al, respectively.^{3,21} Terbinafine is a drug which has gained resistance over a period of time even though it is still the most promising drug for dermatophytoses.^{1,4} We observed values of 4 µg/mL of MIC₉₀ for *T. mentagrophytes* complex and 0.25 µg/mL for *T. rubrum* complex. Most studies have reported values less than this study,^{12,29,36,38} while some studies have values similar to this study.^{3,21} Singh et al have reported quite high MIC₉₀ of 32 µg/mL for terbinafine.²⁸ Itraconazole, in this study, gave the lowest MICs of 0.0625 µg/mL for *T. mentagrophytes* complex and 0.0312 µg/mL for *T. rubrum* complex. These values are in line with most other studies.^{3,10,21,29} Unusually high values of MIC₉₀ of 16 µg/mL for itraconazole have been reported by Badali et al.³⁸

Conclusion

More awareness needs to be generated in the patients of dermatophytosis of skin to seek treatment early in disease so the disease process can be managed before it spreads and

becomes difficult to treat. Early management will also help in containing the spread of infection to other family members. Over the counter availability of fixed dose combinations of ointments containing antifungals and steroids has worsened the situation of dermatophytosis by making them resistant to drugs which should be stopped. More studies based on species distribution and AFST should be performed to understand the changing epidemiology of dermatophytosis.

Authors' Contribution

JC and NG are responsible for conceptualization; MBK, SS, and MK for methodology; MBK and NG for formal analysis and investigation; NG, NS, and CN for writing—original draft preparation; JC and MB for writing—review and editing; and JC for supervision.

Ethical Approval

All procedures performed in the study were in accordance with the ethical standard of the institute research committee and with the 1964 Helsinki declaration.

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None.

Conflicts of Interest

None declared.

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