



Original Article

Clinico-Mycological Profile and Antifungal Susceptibility Pattern of Dermatophytosis in Patients Attending the Dermatology Outpatient Department of a Tertiary Care Hospital: A Cross-Sectional Study

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ABSTRACT

Background: India has seen a sharp rise in chronic, recurrent and treatment-resistant dermatophytosis. Local data on the causative species and their antifungal susceptibility are needed to guide treatment.

Aim: To describe the clinical and mycological profile of dermatophytosis and the in vitro antifungal susceptibility of the isolates in patients attending a dermatology outpatient department.

Materials and methods: This hospital-based cross-sectional study enrolled 150 patients with clinically suspected dermatophytosis of the glabrous skin. Clinical details were recorded on a structured proforma. Skin scrapings were examined by 10% potassium hydroxide (KOH) mount and cultured on Sabouraud dextrose agar with chloramphenicol and cycloheximide. Isolates were identified by colony morphology, microscopy, urease and hair perforation tests. Susceptibility to terbinafine, itraconazole, fluconazole, griseofulvin and voriconazole was tested by broth microdilution following the CLSI M38-A2 standard. Data were analysed with IBM SPSS Statistics 25.0.

Results: The mean age was 29.1 ± 12.9 years, and 59.3% of patients were male. Tinea corporis with cruris was the most common presentation (40.7%). Disease was chronic in 42.0% and recurrent in 44.7%, and 46.7% had used topical corticosteroid creams. KOH mount was positive in 73.3% and culture in 65.3%. Trichophyton mentagrophytes was the predominant isolate (74.5%), followed by Trichophyton rubrum (19.4%). Itraconazole and voriconazole had the lowest MICs (MIC₉₀ 0.25 and 0.125 µg/mL). Terbinafine MIC was 1 µg/mL or higher in 42.9% of isolates, which was significantly associated with previous terbinafine use and chronic disease ($p = 0.023$ and $p < 0.001$).

Conclusion: Trichophyton mentagrophytes was the main cause of dermatophytosis in this setting, and a substantial share of isolates showed raised terbinafine MICs. Mycological confirmation, susceptibility testing in recalcitrant cases and control of topical steroid misuse are needed.

Keywords: Antifungal susceptibility; Dermatophytosis; India; Terbinafine; Tinea corporis; Trichophyton mentagrophytes.

INTRODUCTION

Superficial fungal infections are among the most common infections of humans. An estimated 20% to 25% of the world's population has a skin mycosis, and dermatophytes of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* cause most of them [1]. The species involved vary with geography, climate, living conditions and time. In Central and Northern Europe, *Trichophyton rubrum* has accounted for 80% to 90% of skin isolates since the 1950s, whereas zoophilic species predominate in some Mediterranean and Arab countries [2]. Dermatophytes digest keratin and infect the stratum corneum, hair and nails, and they are identified by their colonies and microscopic features in culture [3].

Over the past decade, India has faced what has been called an epidemic of superficial dermatophytosis [4]. Dermatologists across the country report large numbers of patients with extensive, inflamed, chronic and recurrent tinea corporis and cruris that respond poorly to standard doses of antifungal drugs [5-7]. Several factors are thought to contribute. These include hot and humid weather, tight synthetic clothing, sharing of fomites, poor adherence, and above all the widespread over-the-counter sale and misuse of topical corticosteroid creams, often in irrational fixed-dose combinations with antifungal and antibacterial agents [8].

The spectrum of causative species has also changed. A molecular study of 201 samples from across India found that 92.6% of culture isolates were *Trichophyton mentagrophytes*, with a distinct genotype now called the Indian ITS genotype, and only 7.4% were *T. rubrum* [9]. At the same time, reduced susceptibility to terbinafine has emerged. Point mutations in the squalene epoxidase gene, which encodes the target of terbinafine, have been found in Indian isolates with high terbinafine MICs [10,11], and high MICs have been linked to poor clinical response [12]. In a multicentre study from eight Indian locations, 71% of isolates were resistant to terbinafine [13].

National expert consensus recommends KOH microscopy for all patients and fungal culture for chronic, recurrent, relapsing, recalcitrant and multisite disease [14]. Antifungal susceptibility testing, however, is available in few centres, and patterns differ between regions. There are limited data from western Maharashtra. This study was therefore carried out to describe the clinical and epidemiological profile of dermatophytosis, identify the causative species, and determine the in vitro antifungal susceptibility of the isolates in patients attending the dermatology outpatient department of a tertiary care hospital in Pune.

MATERIALS AND METHODS

Study design and setting

This hospital-based cross-sectional study was conducted in the Department of Skin & VD in collaboration with the Department of Microbiology, Smt. Kashibai Navale Medical College, Pune, a tertiary care teaching hospital. Consecutive eligible patients were enrolled until the calculated sample size was reached.

Ethical considerations

Written informed consent was obtained from all adult participants and from the parents or guardians of participants younger than 18 years, with assent from children aged 7 years or older. Patient identity was kept confidential, and all patients were treated according to the department's standard protocol irrespective of participation.

Sample size

The sample size was calculated with the formula $n = Z^2pq/d^2$. A culture positivity rate of 56.36% was taken from a clinico-mycological study from South India [15], with $Z = 1.96$ for 95% confidence and an absolute precision (d) of 8%. This gave a minimum sample of 148, which was rounded up to 150 patients.

Participants

Consecutive patients of all ages and both sexes with clinically suspected dermatophytosis of the glabrous skin (tinea corporis, tinea cruris, tinea faciei, tinea manuum or tinea pedis) attending the outpatient department were enrolled until the sample size was reached. Patients were excluded if they had used an oral antifungal agent in the previous 4 weeks or a topical antifungal agent in the previous 2 weeks, if they had isolated tinea capitis or onychomycosis without skin involvement, if they had known immunosuppression (HIV infection, malignancy or systemic immunosuppressive therapy), or if they did not consent.

Clinical evaluation

A structured proforma was used to record age, sex, occupation, residence, education, socioeconomic status, family history of similar lesions, duration of disease, number of previous episodes, previous treatment (oral and topical antifungal agents, and topical corticosteroids alone or in fixed-dose combinations), and associated conditions such as diabetes mellitus. A detailed cutaneous examination recorded the clinical type, the number of body sites involved, the approximate body surface area involved, and features of steroid-modified tinea. Disease lasting more than 6 months was defined as chronic, and recurrence of lesions within a few weeks after clinical cure as recurrent, following the national consensus definitions [14].

Sample collection and direct microscopy

The lesion was cleaned with 70% alcohol, and scales were scraped from the active edge with a sterile blunt scalpel onto sterile black paper. Part of each sample was mounted in 10% KOH and examined under the microscope for hyaline, septate, branching fungal hyphae or arthroconidia [16].

Fungal culture and identification

The remaining sample was inoculated onto Sabouraud dextrose agar containing chloramphenicol (0.05 mg/mL) and cycloheximide (0.5 mg/mL), in duplicate, and incubated at 25 °C to 30 °C for up to 4 weeks. Cultures were examined twice weekly and reported as negative if there was no growth at 4 weeks. Dermatophytes were identified by the rate of growth,

colony texture, surface and reverse pigmentation, and microscopic appearance of macroconidia and microconidia in lactophenol cotton blue mounts. Urease test and in vitro hair perforation test were used to separate *T. mentagrophytes* from *T. rubrum* [3].

Antifungal susceptibility testing

Antifungal susceptibility of the dermatophyte isolates was tested by broth microdilution according to the Clinical and Laboratory Standards Institute document M38-A2 (2008), using a method validated for dermatophytes [17]. Isolates were subcultured on potato dextrose agar to induce conidiation. Conidial suspensions were adjusted to a final inoculum of 1×10^3 to 3×10^3 CFU/mL in RPMI 1640 medium buffered with MOPS. The drugs tested and their concentration ranges were terbinafine (0.03 to 16 µg/mL), itraconazole (0.03 to 16 µg/mL), voriconazole (0.03 to 16 µg/mL), fluconazole (0.125 to 64 µg/mL) and griseofulvin (0.125 to 64 µg/mL). Plates were incubated at 35 °C and read after 4 days. The MIC was the lowest concentration giving 80% inhibition of growth compared with the drug-free control. *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258 and *Trichophyton mentagrophytes* ATCC MYA-4439 were used as quality control strains. Because clinical breakpoints for dermatophytes have not been established, a terbinafine MIC of 1 µg/mL or higher was regarded as elevated, as in an earlier Indian study [12].

Statistical analysis

Data were entered in Microsoft Excel and analysed with IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA, 2017). Categorical variables are presented as numbers and percentages, and continuous variables as mean $\pm$ standard deviation or median and interquartile range. For each drug, the MIC range, MIC₅₀, MIC₉₀ and geometric mean MIC were calculated. Agreement between KOH mount and culture was assessed with Cohen's kappa. The association of clinical factors (duration, recurrence, previous terbinafine use and topical corticosteroid use) with elevated terbinafine MIC was tested with the chi-square test or Fisher's exact test. A p value below 0.05 was considered statistically significant.

RESULTS

Sociodemographic profile

A total of 150 patients were enrolled. Their mean age was 29.1 ± 12.9 years (range 3 to 63 years), and the largest group was aged 21 to 30 years (35.3%). There were 89 males (59.3%) and 61 females (40.7%), a male-to-female ratio of 1.46:1, and 59.3% lived in urban areas. Office workers and others (34.0%) and students (31.3%) formed the largest occupational groups. A family history of similar lesions was present in 26.7% of patients (Table 1).

Table 1: Sociodemographic characteristics of the study participants (n = 150)

Characteristic	Category	n	%
Age group (years)	Up to 10	6	4.0
	11 to 20	30	20.0
	21 to 30	53	35.3
	31 to 40	33	22.0
	41 to 50	18	12.0
	Above 50	10	6.7
Sex	Male	89	59.3
	Female	61	40.7
Residence	Urban	89	59.3
	Rural	61	40.7
Occupation	Student	47	31.3
	Homemaker	10	6.7
	Manual or outdoor worker	42	28.0
	Office worker or other	51	34.0
Family history of similar lesions	Present	40	26.7
Diabetes mellitus	Present	20	13.3

Clinical profile

The median duration of disease was 5.2 months (interquartile range 2.8 to 9.1). Tinea corporis with tinea cruris was the most common presentation (40.7%), followed by tinea corporis alone (24.0%) and tinea cruris alone (15.3%). Multiple sites were involved in 56.0% of patients. Disease was chronic in 42.0% and recurrent in 44.7%. Previous use of a topical corticosteroid, alone or in a fixed-dose combination, was reported by 46.7% of patients, most of whom had bought it

without a prescription (77.1%). Oral terbinafine had been taken before by 28.7% of patients and oral itraconazole by 14.7%. Features of steroid-modified tinea were seen in 23.3% (Table 2).

Table 2: Clinical profile of the study participants (n = 150)

Variable	Category	n	%
Clinical type	Tinea corporis with cruris	61	40.7
	Tinea corporis	36	24.0
	Tinea cruris	23	15.3
	Tinea faciei	15	10.0
	Tinea manuum or pedis	15	10.0
Number of sites	Single	66	44.0
	Multiple	84	56.0
Duration	6 months or less	87	58.0
	More than 6 months (chronic)	63	42.0
Recurrent disease	Yes	67	44.7
Previous topical corticosteroid use	Yes	70	46.7
Previous oral terbinafine	Yes	43	28.7
Previous oral itraconazole	Yes	22	14.7
Steroid-modified tinea	Present	35	23.3

Direct microscopy and culture

KOH mount was positive in 110 of 150 samples (73.3%), and culture was positive in 98 (65.3%). Both methods were positive in 74 samples (49.3%), KOH alone in 36 (24.0%), culture alone in 24 (16.0%) and neither in 16 (10.7%). Agreement between KOH mount and culture was only slight ($\kappa = 0.07$; $p = 0.48$) (Table 3).

Table 3: Comparison of KOH mount and fungal culture (n = 150)

KOH mount	Culture positive, n (%)	Culture negative, n (%)	Total, n (%)
Positive	74 (49.3)	36 (24.0)	110 (73.3)
Negative	24 (16.0)	16 (10.7)	40 (26.7)
Total	98 (65.3)	52 (34.7)	150 (100)

KOH: potassium hydroxide. Cohen's $\kappa = 0.07$ ($p = 0.48$).

Species distribution

Of the 98 dermatophyte isolates, 73 (74.5%) were *Trichophyton mentagrophytes*, 19 (19.4%) were *Trichophyton rubrum*, 2 (2.0%) were *Trichophyton tonsurans*, 2 (2.0%) were *Epidermophyton floccosum* and 2 (2.0%) were *Microsporum gypseum*. *T. mentagrophytes* was the predominant isolate in tinea corporis with cruris (28 of 39, 71.8%) and in chronic or recurrent cases (55 of 72, 76.4%) (Table 4).

Table 4: Distribution of dermatophyte species by clinical type (n = 98 isolates)

Species	Tinea corporis with cruris	Tinea corporis	Tinea cruris	Other sites	Total, n (%)
<i>Trichophyton mentagrophytes</i>	28	18	11	16	73 (74.5)
<i>Trichophyton rubrum</i>	7	5	3	4	19 (19.4)
<i>Trichophyton tonsurans</i>	0	1	0	1	2 (2.0)
<i>Epidermophyton floccosum</i>	2	0	0	0	2 (2.0)
<i>Microsporum gypseum</i>	2	0	0	0	2 (2.0)
Total	39	24	14	21	98 (100)

Other sites: tinea faciei, tinea manuum and tinea pedis.

Antifungal susceptibility

Antifungal susceptibility testing was done on 98 isolates (Table 5). Itraconazole and voriconazole had the lowest MICs, with MIC₉₀ values of 0.25 $\mu\text{g/mL}$ and 0.125 $\mu\text{g/mL}$ and geometric mean MICs of 0.066 $\mu\text{g/mL}$ and 0.057 $\mu\text{g/mL}$. Terbinafine MICs ranged from 0.03 to 8 $\mu\text{g/mL}$, with an MIC₅₀ of 0.25 $\mu\text{g/mL}$ and an MIC₉₀ of 4 $\mu\text{g/mL}$. Fluconazole and griseofulvin had the highest MICs. A terbinafine MIC of 1 $\mu\text{g/mL}$ or higher was found in 42 isolates (42.9%), 36 of

which were *T. mentagrophytes*. Elevated terbinafine MIC was significantly more common in patients with previous oral terbinafine use (62.1% vs 34.8%; $p = 0.023$) and chronic disease (61.7% vs 25.5%; $p < 0.001$), but not in those with previous topical corticosteroid use ($p = 0.255$).

Table 5: In vitro antifungal susceptibility of dermatophyte isolates (n = 98)

Antifungal agent	MIC range ($\mu\text{g/mL}$)	MIC50 ($\mu\text{g/mL}$)	MIC90 ($\mu\text{g/mL}$)	Geometric mean MIC ($\mu\text{g/mL}$)
Terbinafine	0.03 to 8	0.25	4	0.345
Itraconazole	0.03 to 0.5	0.06	0.25	0.066
Voriconazole	0.03 to 0.5	0.06	0.125	0.057
Fluconazole	0.5 to 32	4	16	4.173
Griseofulvin	0.25 to 16	2	8	2.208

MIC: minimum inhibitory concentration; MIC50 and MIC90: MICs inhibiting 50% and 90% of isolates.

DISCUSSION

This study describes the clinical and mycological profile of dermatophytosis and the antifungal susceptibility of the isolates in a tertiary care hospital in Pune. Young adults and males were most often affected, tinea corporis with cruris was the commonest presentation, and a large proportion of patients had chronic or recurrent disease and had used topical corticosteroids. *Trichophyton mentagrophytes* was the main species, and a substantial proportion of isolates had raised terbinafine MICs.

The predominance of young adults and males in our study is consistent with earlier Indian reports. In a tertiary care hospital in North Karnataka, the commonest age group was 21 to 30 years and the male-to-female ratio was 1.63:1 [18]. In Himachal Pradesh, most patients were aged 21 to 50 years and males far outnumbered females [19]. The clinico-mycological profile of dermatophytosis has also been documented at a tertiary care centre in Northwest India [20]. Greater outdoor activity, sweating, occlusive clothing and occupational exposure may explain the higher rates in young men. The 46.7% rate of previous topical corticosteroid use in our patients reflects the easy over-the-counter availability of steroid-containing creams in India [4,8].

KOH mount was positive in 73.3% and culture in 65.3% of our samples. These rates are higher than the 58.18% and 56.36% reported from South India [15], close to the 74.13% culture positivity in a multicentre Indian study [9], and well above the 36.6% culture yield from Himachal Pradesh [19]. In patients with recalcitrant tinea in Delhi, KOH mount was positive in only 45% while culture was positive in 70% [21], which shows that KOH-negative samples should still be cultured in chronic and recurrent cases. In our study, 24 of the 40 KOH-negative samples (60.0%) grew a dermatophyte, and agreement between the two tests was only slight.

T. mentagrophytes was the predominant species in our study (74.5%). This agrees with the clear shift in species reported from across India in recent years. Earlier studies found *T. rubrum* to be the commonest isolate, accounting for 79% of dermatophytes in rural Tamil Nadu [22] and 58.06% in South India [15], and a change in the spectrum of dermatophytes was reported from Central India as early as 2011 [23]. In contrast, *T. mentagrophytes* accounted for 63.5% of isolates in Himachal Pradesh [19], 40% of isolates from recurrent dermatophytosis in Chandigarh [24], 92.6% of isolates in a pan-Indian molecular study [9] and 78% of samples in a multicentre study from eight locations [13]. The Indian ITS genotype of *T. mentagrophytes* appears well adapted to spread from person to person and may have been favoured by widespread steroid misuse [9].

Itraconazole and voriconazole showed the best in vitro activity in our study, which supports the current preference for itraconazole in extensive and recalcitrant disease [14]. Terbinafine MICs of 1 $\mu\text{g/mL}$ or higher were found in 42.9% of our isolates. Reported rates vary widely across India. In Delhi, 61% of *T. interdigitale* isolates from tinea corporis and cruris had terbinafine MICs of 1 $\mu\text{g/mL}$ or higher, and these high MICs were linked to poor clinical response [12]. In the multicentre study, 71% of isolates were resistant to terbinafine, and 91% of the resistant *T. mentagrophytes* isolates carried the Phe397Leu substitution in squalene epoxidase [13]. Other Delhi and Chandigarh studies found terbinafine-resistant isolates with MICs of 4 to 32 $\mu\text{g/mL}$ or more, all with point mutations in the squalene epoxidase gene [10,11]. By contrast, another Delhi study of recalcitrant cases found low terbinafine MIC90 values of 0.125 $\mu\text{g/mL}$ [21]. Such differences may reflect local drug pressure, the populations studied and differences in laboratory methods, which is why each centre needs its own data.

The association of elevated terbinafine MIC with previous terbinafine use and chronic disease suggests that repeated, short or low-dose courses may select for less susceptible strains. In Kashmir, only 43% of patients with tinea corporis or cruris achieved long-term clinical and mycological cure after 2 weeks of oral terbinafine, and many relapsed within 12 weeks

[25]. Treatment failure, however, is not explained by resistance alone. Poor adherence, inadequate duration, reinfection from family members and fomites, and continued use of topical steroids all contribute [5,6].

These findings have practical implications. Mycological confirmation should be the rule before starting systemic therapy, and culture with susceptibility testing should be done in chronic, recurrent and recalcitrant cases [14]. The sale of topical corticosteroid combinations without prescription must be controlled. Patients and families should be counselled about adherence, treatment duration, hygiene and the treatment of affected household contacts.

Strengths and limitations

The strengths of this study are its consecutive enrolment, the use of both direct microscopy and culture, and standardised broth microdilution testing with quality control strains. It also has limitations. It was done in a single tertiary centre, so the results may not apply to primary care. Species were identified by conventional methods, and molecular identification and squalene epoxidase gene sequencing were not done. Clinical breakpoints for dermatophytes are not established, so in vitro MICs cannot be translated directly into clinical resistance. Finally, patients were not followed up to correlate MICs with treatment response.

CONCLUSION

Dermatophytosis in this tertiary care setting mainly affected young adults and presented most often as extensive tinea corporis with cruris, frequently chronic or recurrent and often modified by topical steroid misuse. Trichophyton mentagrophytes was the main causative species. Itraconazole and voriconazole showed the best in vitro activity, while a substantial share of isolates had raised terbinafine MICs. Routine mycological confirmation, periodic local susceptibility surveillance and strict control of over-the-counter topical steroids are needed.

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Conflicts of interest

There are no conflicts of interest.

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