A Study of In vitro Antifungal Susceptibility Patterns of Dermatophytic Fungi at a Tertiary Care Center in Western India

Shital Poojary, Autar Miskeen1, Jimish Bagadia, Saurabh Jaiswal, Priya Uppuluri2

Abstract

Background: Recent years have seen an alarming rise in the prevalence of recalcitrant and relapsing dermatophyte infections in India associated with lack of clinical response to standard antifungal regimens. Aims and Objectives: A study was undertaken to identify the antifungal susceptibility patterns of dermatophyte species isolated from lesions of dermatophytoses in patients examined at our center. Materials and Methods: A total of 85 patients with clinically diagnosed dermatophytoses were subjected to skin scrapings for potassium hydroxide mount (microscopic examination) and culture using Sabouraud's agar medium containing chloramphenicol and cycloheximide (incubated at 30°C). Antifungal susceptibilities [minimum inhibitory concentration-90 (MIC-90)] of the identified dermatophytes were tested for seven systemic and topical antifungal agents (terbinafine, griseofulvin, itraconazole, fluconazole, sertaconazole, ketoconazole, and clotrimazole) using Clinical and Laboratory Standards Institute broth microdilution method (M38-A). Results: Trichophyton rubrum (50%) and Trichophyton mentagrophytes complex (47.2%) were the two major species isolated. Isolates of both showed downy and granular forms (61.11%, 38.89% and 32.35%, 67.65%, respectively). The overall in-vitro susceptibility profiles (MIC-90 ranges in µg/mL) of the seven drugs for T. rubrum and T. mentagrophytes complex respectively were as follows: terbinafine (0.008–0.256, 0.016–0.256), griseofulvin (0.03-1, 0.06–1), itraconazole (0.125–2, 0.25–2), fluconazole (0.125–1, 0.25–32), sertaconazole (0.03-1, 0.03-1), ketoconazole (0.06–1, 0.125–1), and clotrimazole (0.03–2, 0.06–1). Conclusions: This study indicates a rising proportion of T. mentagrophytes complex with increased proportion of granular form (T. mentagrophytes var. mentagrophytes). This study represents the current antifungal susceptibility profile of dermatophytic infections in a tertiary care medical center in western India with rising MICs to terbinafine and itraconazole.

Key Words: Antifungal susceptibility, dermatophytes, minimum inhibitory concentration

Introduction

Within the past 5 years, there has been a sudden, unexplained surge in dermatophytoses in India.[1] This epidemic has been characterized by recurrent, recalcitrant infections characterized by unusual clinical patterns including erythrodermic dermatophytoses, pseudomibricata patterns, and scalp involvement in adults.[10] These patterns of dermatophyte infections have been observed even in patients who have been compliant with standard antifungal therapies. However, there are very few studies documenting the present clinicoepidemiological patterns of dermatophytoses in India. More importantly, there are even fewer studies outlining antifungal susceptibility of common dermatophyte species in India. The paucity of epidemiological data, coupled with so far undetermined breakpoint minimum inhibitory concentration (MIC) values of antifungals against dermatophytes adds further to the difficulty in managing the current dermatophyte epidemic.

Hence, the present work was conducted to study the antifungal susceptibility patterns of dermatophytes isolated from lesions of dermatophyte infections at our tertiary care medical facility in western India.

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Aims and Objectives
1. To identify the causative dermatophyte species responsible for dermatophytoses in patients reporting to the tertiary care center.
2. To determine the in-vitro MIC-90 of these dermatophyte species to seven antifungal agents: terbinafine, griseofulvin, itraconazole, sertaconazole, fluconazole, ketoconazole, and clotrimazole.

Materials and Methods
A total of 85 clinically diagnosed cases of dermatophytoses were included in this cross-sectional study. The study was conducted over a period of 1 year in a tertiary health care center of western India after institutional ethics committee approval. Their demographic details and clinical examination of cutaneous lesions were recorded.

Inclusion criteria
All clinically diagnosed cases of dermatophytoses.

Exclusion criteria
1. Any patient treated with oral/topical antifungals within 1 week prior to inclusion in the study
2. Any patient with suspected deep dermatophytoses
3. Any patient with secondary infection of dermatophytic lesions.

Mycology laboratory procedures
Scraping of the skin from the active border of the lesions was collected and transported under sterile conditions to the microbiology laboratory. In cases of tinea capitis, hair mount was taken and in tinea unguium, nail clippings were taken. Microscopic examination for the presence of fungi was performed after treatment of an aliquot of the skin scales with 10% potassium hydroxide (KOH). All specimens were also inoculated on Sabouraud’s agar medium containing chloramphenicol and cycloheximide. These inoculated media plates were incubated at 30°C with daily observation for fungal colonies. Slide microcultures and lactophenol cotton blue mount were prepared for observing microscopic morphology of the fungal colonies. Species identification of dermatophytes was done on basis of gross and microscopic morphological characteristics including their pattern of conidiation and physiological tests (urease test and in-vitro hair perforation test).

Antifungal susceptibility testing
In-vitro antifungal susceptibility assay was performed by following the Clinical and Laboratory Standards Institute (CLSI) guidelines, document M38-A2 for filamentous fungi. Dermatophytes were subcultured from the primary Sabouraud’s agar plate to oat meal agar medium to induce conidiation. Plates were incubated at 35°C for 7 days or longer until colonies developed abundant spores. The conidial spores were then carefully collected by gently flushing 5 ml phosphate buffer saline (pH = 7.4) on top of the colonies and aspirating the suspension into a sterile collection tube. The fungal spores were enumerated using a hemocytometer and cell numbers adjusted to 1 × 10^6 cells/ml. Susceptibility of isolates to seven antifungals was tested in the study; itraconazole, griseofulvin, and fluconazole were the systemic antifungals, while sertaconazole and clotrimazole were the topical antifungals. Ketoconazole and terbinafine are being used both as oral and topical preparations in India. The antifungal drug powders (terbinafine, griseofulvin, itraconazole, ketoconazole, fluconazole, clotrimazole, and sertaconazole) were purchased from Sigma-Aldrich. Stock solution of all drugs was prepared in dimethyl sulfoxide, at a concentration of 2 mg/ml (except fluconazole, which was dissolved in water).

Next, the drug stock solutions were diluted in Roswell Park Memorial Institute 1640 liquid medium (RPMI) buffered with MOPS (3-N-Morpholinopropanesulfonic acid), in twice the final concentration followed by addition of equal volume of the preadjusted inoculum of dermatophyte isolates, in 96-well microtiter plates. The following final concentration ranges were tested: terbinafine (0.004–1.0 µg/ml), griseofulvin (0.03–4.0 µg/mL), itraconazole (0.06–32 µg/ml), ketoconazole (0.06–32 µg/ml), sertaconazole (0.007–4.0 µg/ml), fluconazole (0.125–64 µg/ml), and clotrimazole (0.06–16 µg/ml). All plates were incubated at 35°C for 4 days or longer, until sufficient growth (i.e., confluent hyphal growth covering the bottom of the well) was observed in control wells containing media without antifungal drug. T. mentagrophytes ATCC MYA-4439 was chosen as a quality control strain and an in-house T. rubrum strain displaying consistently reproducible susceptibilities to MICs of all antifungals tested were also used for comparative control purposes. MIC-90 (i.e., the concentration of the drug that inhibits 90% of growth of the fungus.) of the drug against the fungus in each well was detected visually by using a magnifying reading glass. The reading was taken at 4 days or until the day of sufficient growth of the fungus in the control well for comparison. Slides microscopy and culture identification by growth characteristics, pigment formation, and lactophenol cotton blue mount were used as criteria for nondermatophytes isolation.

Statistical analysis was performed by Mann-Whitney U test and Kruskal-Wallis tests using SPSS-16 software in order to find the significant differences between variables. P value of <0.05 was considered significant.

Results
Of the 85 patients diagnosed with dermatophytoses and included in the study, there were 41 females (48.2%) and 44 males (51.8%). Age range of the patients was 2–72 years (mean age: 30.42 ± 14.59). Extensive
dermatophytic infection (lesions involving >20% body surface area or ≥ two noncontiguous sites) was present in 41 cases (48.23%) [Figure 1], while 44 cases (51.76%) had localized lesions. Among the 85 cases, co-occurrence of tinea corporis and tinea cruris (32 cases) was most common, followed by only tinea corporis (30 cases) and tinea cruris (10 cases). Face was involved in seven cases, whereas nail and scalp were the least common sites (5 and 1, respectively) [Table 1].

**Microscopy and culture characteristics of dermatophytic fungi isolated from patient specimens**

Out of 85 samples, 76 samples (89.4%) showed hyphae and spores on KOH examination, and 72 samples were culture positive (84.7%). Scrapings were taken from multiple sites so as to improve the culture outcomes and also to determine if different species cohabitated in one patient at a time.

There was an abundance of hyaline, long, smooth, septate, branching hyphae on KOH mounts of 72.36% of the specimens [Figure 2].

*T. rubrum* (*n* = 36, 50%) and *T. mentagrophytes complex* (*n* = 34, 47.22%) were the predominant species identified with an almost equal frequency of occurrence; whereas *T. violaceum* was isolated in one patient of tinea capitis (1.39%). *Acremonium* was isolated from one suspected case of onychomycosis [Figures 3, 4 and Table 2].

Isolates of *T. rubrum* and *T. mentagrophytes* showed two distinctive types of colony forms [Table 2 and Figures 3, 4]

- Downy form: Slow-growing colonies with velvety surface
- Granular form: Rapidly growing (within a week) powdery/chalky colonies.

**Antifungal susceptibility profiles of the isolated dermatophytic fungi**

MIC-90 values were obtained for all the isolates of dermatophytes (single case of *Acremonium* isolate was excluded). *In vitro* susceptibility (MIC-90 ranges and median MIC-90) of seven oral and topical antifungal agents, against the isolated dermatophyte species is illustrated in Tables 3 and 4.

MIC-90 levels of the single isolate of *T. violaceum* were as follows: terbinafine-0.128 µg/ml, griseofulvin-0.24 µg/ml, and ciclopirox-0.012 µg/ml.

| Clinical presentation       | (n) | KOH +ve | KOH -ve | Culture +ve | Culture -ve | TR | TM | Others | TRD | TRG | TMD | TMG |
|----------------------------|-----|---------|---------|-------------|-------------|----|----|--------|-----|-----|------|------|
| *T. corporis*              | 30  | 27      | 3       | 27          | 3           | 10 | 17 | -      | 4   | 6   | 3    | 14   |
| *T. cruris*                | 10  | 8       | 2       | 7           | 3           | 5  | 2  | -      | 1   | 4   | 1    | 1    |
| Onychomycosis              | 3   | 2       | 1       | 2           | 1           | 1  | -  | 1      | 1   | -   | -    | -    |
| *T. capitis*               | 1   | 1       | -       | 1           | -           | -  | -  | 1      | -   | -   | -    | -    |
| *T. corporis + T. cruris*  | 32  | 29      | 3       | 26          | 6           | 15 | 11 | -      | 12  | 3   | 4    | 7    |
| *T. corporis + T. faciei*  | 4   | 4       | -       | 4           | -           | 2  | 2  | -      | 1   | 1   | 2    | 1    |
| *T. cruris + T. faciei*    | 1   | 1       | -       | 1           | -           | -  | -  | 1      | -   | 1   | -    | 1    |
| *T. corporis + T. cruris + T. faciei* | 2 | 2 | - | 2 | 1 | 1 | - | - | 1 | - | - | - |
| *T. corporis + T. cruris + onychomycosis* | 1 | 1 | - | 1 | - | 1 | - | - | 1 | - | - | - |
| *T. cruris + onychomycosis* | 1   | 1       | -       | 1           | -           | 1  | -  | -      | 1   | -   | -    | -    |

TR: Trichophyton rubrum; TM: *T. mentagrophytes complex*, TRD: Trichophyton rubrum, downy form, TRG: *T. rubrum*, granular form, TRD: *T. mentagrophytes*, downy form, TMG: *T. mentagrophytes*, granular form

**Table 1: Various clinical presentations along with KOH and culture results**

**Figure 1:** Extensive lesions of Tinea corporis in a middle-aged female patient

**Figure 2:** KOH mount: Showing abundant hyaline branching septate hyphae and chains of arthroconidia (×200)
Statistical analysis

Comparison of median MIC-90 for the isolated dermatophyte species:

i) T. rubrum vs. T. mentagrophytes complex

Median MIC-90 values for itraconazole were significantly higher in T. mentagrophytes complex group as compared to T. rubrum group (\(P<0.01\), Mann–Whitney U test).

The median MIC-90 was again higher in the T. mentagrophytes complex group for terbinafine (0.096 vs. 0.064) and ketoconazole (0.5 vs. 0.25), whereas T. rubrum group showed higher median MIC-90 for fluconazole (1.0 vs. 0.5) and clotrimazole (0.25 vs. 0.125); however in each of these, the difference was not statistically significant. The MICs for griseofulvin and sertaconazole were similar for both the groups.

ii) Comparison of downy vs. granular forms of T. rubrum

The median MIC-90 values were higher in the T. rubrum, granular form for terbinafine and clotrimazole as compared to T. rubrum, downy form, while the latter showed higher MICs for fluconazole and griseofulvin. The differences were however not statistically significant.

iii) Comparison of downy vs. granular forms of T. mentagrophytes:

T. mentagrophytes, downy form showed higher median MIC-90 than granular form for terbinafine and sertaconazole. The differences among both the groups were however not statistically significant.

iv) Comparison of all four subspecies (granular and downy forms of T. rubrum and T. mentagrophytes) for individual drug susceptibilities (Kruskal–Wallis test): showed highest MIC-90 for T. mentagrophytes downy group for itraconazole (\(P < 0.05\)), terbinafine, sertaconazole, and ketoconazole. T. rubrum downy group showed highest MIC-90 for griseofulvin and fluconazole. T. rubrum, granular form showed highest MIC-90 for clotrimazole. These differences were not statistically significant for any of the antifungals, except for itraconazole in T mentagrophytes downy group.

Discussion

The recent changing clinicoepidemiological scenario of dermatophyte infections in India has presented a challenge for identifying the factors responsible for this abrupt change. The rising trend of recalcitrant dermatophytoses could possibly be due to some or all of the followings: a) an epidemiological shift in the
growth patterns of dermatophytes providing them with an advantage for better persistence in the human host, b) an evolution in the genetic make-up of the fungi that perhaps enhances its virulence traits and supports pathogenicity, c) a rapid emergence in the drug resistant species perhaps due to rampant use of potent antifungal drugs in inadequate doses, and d) human host factors. Obesity (due to changing food habits), climate change (longer exposure to the hot and humid climate), change in clothing habits (tight, synthetic material), and most importantly topical steroid misuse help in persistence of the fungi in its colonized niches.

Our study has confirmed the recent increase in extensive forms of the disease with 48.23% of the patients having extensive dermatophytosis. The clinical pattern paralleled results from the mycological examination of the specimens with almost three fourths of fungal KOH mounts showing an overabundance of long branched intertwined dermatophytic hyphae clearly indicating a higher fungal load at the site of infection. This picture was reminiscent of a biofilm-like phenotype which is a concentrated community of organisms that build up on each other and develop vast amounts of hyphae in the niche. This microscopic picture is in stark contrast to that observed perhaps only a decade ago, wherein dermatophytic hyphae were found only after laboriously scanning several fields of one or more KOH mounts.

The changing scenario in the epidemiological growth pattern of dermatophytic fungi was additionally reflected in its growth phenotype on culture; almost half of the infections in the study were caused by *T. mentagrophytes* complex. Historically in India, and worldwide, *T. rubrum* used to be the primary pathogen isolated from cases of dermatophytoses. In contrast, some of the recent studies have revealed an augmenting prevalence of *T. mentagrophytes* complex. Thus, the recent explosive expansion of superficial dermatophytosis in India seems to have its base over a perceptible epidemiological shift in species, with an increasing proportion of *T. mentagrophytes* complex.

An interesting trend seen on culture growth was the preponderance of rapidly growing granular form, especially in case of *T. mentagrophytes* complex. Normal time required for growth of dermatophyte species on culture is at least 2 weeks. The colonies of these granular forms however grew extremely rapidly within

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**Table 3: Susceptibility data (MIC-90 ranges) for *T. rubrum* (downy and granular), *T. rubrum* (downy), *T. rubrum* (granular), *T. mentagrophytes* (downy and granular forms), *T. mentagrophytes* (downy) and *T. mentagrophytes* (granular)**

| MIC 90 range (µg/mL) | TR       | TR colony types | TM       | TM colony types | Overall |
|---------------------|----------|-----------------|----------|-----------------|--------|
|                     |          | Downy | Granular | Downy | Granular |         |
| TRB                 | 0.008-0.256 | 0.008-0.256 | 0.016-0.256 | 0.016-0.256 | 0.008-0.256 |
| GRF                 | 0.03-1  | 0.03-1 | 0.12-0.5 | 0.06-1 | 0.06-1 | 0.03-1 |
| ITZ                 | 0.125-2 | 0.125-2 | 0.125-1 | 0.25-2 | 0.25-2 | 0.125-2 |
| FLC                 | 0.125-1 | 0.125-1 | 0.5-1 | 0.25-32 | 0.25-32 | 0.125-32 |
| STC                 | 0.03-1  | 0.03-0.5 | 0.03-1 | 0.03-1 | 0.03-1 | 0.06-1 |
| KTC                 | 0.06-1  | 0.06-1 | 0.06-1 | 0.125-1 | 0.125-1 | 0.06-1 |
| CTZ                 | 0.03-2.0 | 0.03-2.0 | 0.125-0.5 | 0.06-1 | 0.06-1.0 | 0.03-2.0 |

TR: Terbinafine, GRF: Griseofulvin, ITZ: Itraconazole, FLC: Fluconazole, STC: Sertaconazole, KTC: Ketoconazole, CTZ: Clotrimazole, TR: *T. rubrum*, TM: *T. mentagrophytes*

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**Table 4: Susceptibility data (Median MIC-90) for *T. rubrum* (downy and granular), *T. rubrum* (downy), *T. rubrum* (granular), *T. mentagrophytes* (downy and granular forms), *T. mentagrophytes* (downy) and *T. mentagrophytes* (granular)**

| Median MIC 90 (µg/mL) | TR       | TR colony types | TM       | TM colony types |
|----------------------|----------|-----------------|----------|----------------|
|                      |          | Downy | Granular | Downy | Granular |
| TRB                  | 0.064   | 0.032 | 0.064 | 0.096 | 0.128 |
| GRF                  | 0.24    | 0.24 | 0.18 | 0.24 | 0.24 |
| ITZ                  | 0.5     | 0.5 | 0.5 | 0.5 | 0.5 |
| FLC                  | 1       | 0.5 | 0.5 | 0.5 | 0.5 |
| STC                  | 0.06    | 0.06 | 0.06 | 0.06 | 0.125 |
| KTC                  | 0.25    | 0.25 | 0.25 | 0.5 | 0.5 |
| CTZ                  | 0.25    | 0.125 | 0.25 | 0.125 | 0.125 |

TR: Terbinafine, GRF: Griseofulvin, ITZ: Itraconazole, FLC: Fluconazole, STC: Sertaconazole, KTC: Ketoconazole, CTZ: Clotrimazole, TR: *T. rubrum*, TR: *T. mentagrophytes*
a week. *T. rubrum* and *T. mentagrophytes* are known to show polymorphic growth of colonies. Downy form is known to be associated with chronic human infections, while granular form is associated with animal infections and acute inflammatory human infections.\[11\] Downy and granular forms of *T. mentagrophytes* are designated as *T. mentagrophytes var. interdigitale* and *T. mentagrophytes var. mentagrophytes*, respectively.\[12\] Downy form of the *Trichophyton* spp. used to be commonly found in the past. Rapid growth of granular forms of dermatophytes correlates with the recent clinical observations of rapidly spreading clinical lesions as well as rapid reappearance of lesions after clearance on therapy.

It is evident from Table 5 that recent Indian studies by Indira and Bhatia and Sharma show the MIC-90 ranges to be slightly higher in *T. mentagrophytes* as compared to *T. rubrum* for terbinafine.\[13,14\] Studies by Indira and Sowmya et al. showed higher MIC-90 ranges for *T. mentagrophytes* to griseofulvin and fluconazole, respectively.\[13,15\] The study by Sowmya et al. however shows *T. rubrum* to have higher MIC-90 ranges for itraconazole.\[15\] Our study showed MIC-90 (median) of itraconazole to be significantly higher in *T. mentagrophytes complex* as compared to *T. rubrum* (P<0.01, Mann–Whitney U test). Though this difference in susceptibility of the two species is variable across antifungals and also the regions involved, it may represent a trend of higher MIC-90 levels of *T. mentagrophytes* vs. *T. rubrum* in India.

While comparing this observation with two similar studies from a different tropical country (Brazil), no such difference in MIC-90 of *T. rubrum* and *T. mentagrophytes* is seen for terbinafine, while *T. rubrum* shows higher MIC-90 range for itraconazole in the study by Rodrigue et al.\[16,17\]

Since there are no specified breakpoints for antifungal susceptibility in dermatophytes, we compared the MICs in our study to similar studies in India as well as in other countries for *T. rubrum* and *T. mentagrophytes*.

### Table 5: Comparative susceptibility data MIC-90 ranges (µg/mL) of *T. rubrum* and *T. mentagrophytes* from studies in India and other countries

| Studies                          | *T. rubrum* | *T. mentagrophytes* |
|---------------------------------|-------------|---------------------|
|                                 | TRB     | GRS    | ITR | FLU | KTZ | TRB     | GRS   | ITR | FLU | KTZ |
| Indira,\[13\] south India, 2014 | 0.001-0.08 | 0.16-5.12 | 0.03-3.84 | 0.16-20.48 | 0.01-3.84 | **0.002-0.16** | **0.32-5.12** | 0.03-1.92 | 0.08-20.48 | 0.01-0.96 |
| Sowmya et al.,\[15\] south India, 2015 | 0.001-0.008 | 0.25-0.5 | - | 0.125-2 | 0.03-1 | 0.001-0.008 | 0.25-0.5 | - | **1.0-8** | **0.25-1.0** |
| Bhatia and Sharma,\[14\] north India, 2015 | 0.03-4 | - | 0.015-1 | - | 0.015-0.5 | **0.06-4** | - | 0.015-1 | - | 0.015-2 |
| Dabas et al.,\[19\] north India, 2017 | 0.03-8 | 1.0-8 | 0.03-0.06 | - | - | 0.03-16 | 0.03-16 | 0.03- | - | - | 0.125 |
| Pathania et al.,\[20\] north India, 2017 | 0.015-16 | 0.5-128 | 0.03-0.5 | 0.03-16 | - | 0.015-8 | 0.5-128 | 0.015-1 | **0.12-32** | - |
| Present study, western India, 2016 | 0.008-0.256 | 0.03-1 | 0.125-2 | 0.125-1 | 0.06-1 | **0.016-0.256** | **0.06-1** | **0.25-2** | **0.25-32** | **0.125-1** |
| Santos and Hamdan,\[18\] Brazil, 2005 | <0.031 | 0.25-2 | <0.031-0.5 | 2-32 | 0.031-2 | - | - | - | - | - |
| Rodrigues et al.,\[16\] Brazil, 2006 | 0.03-0.5 | 0.25-2 | 0.03-4 | 2.0-32 | 0.03-4 | 0.03-0.5 | 0.25-1 | 0.03-0.25 | 4.0-16 | 0.03-1 |
| Barros et al.,\[17\] Brazil, 2006 | <0.007-0.031 | 0.062-1 | 0.015-0.25 | 1.0-64 | - | <0.007-0.031 | 0.062-1 | 0.015-0.25 | 1.0-64 | - |

TRB: Terbinafine, GRS: Griseofulvin, ITR: Itraconazole, FLU: Fluconazole, KTZ: Ketoconazole
other tropical countries. The present study showed higher MIC-90 ranges for terbinafine for both T. rubrum and T. mentagrophytes complex in comparison with those in studies by Indira and Sowmya et al.; also MIC-90 ranges in present study were several folds higher than those in the study by Barros et al. and Santos and Hamdan. The studies by Bhatia and Sharma and Dabas et al. showed MIC-90 ranges to terbinafine to be even higher than in our study.4,19

On the contrary, MIC-90 ranges for fluconazole were higher in the study by Pathania et al. than in the present study, while those for griseofulvin were higher in the study by Indira in comparison with our study.13,20 MIC-90 ranges for itraconazole were higher than those in studies by Bhatia and Sharma and Pathania et al.4,19

From these observations, we can infer that antifungal susceptibilities vary widely with geographical attributes even with identical testing methods. There is however an evident trend of rising MIC-90 to terbinafine in India though a clear statement about resistance to terbinafine cannot be made due to lack of CLSI guidelines defining the drug breakpoints. This emphasizes the need for several such studies from different regions in India along with in vivo correlation to define the MIC breakpoints. Recent Indian reports of mutation in squalene epoxidase gene in Trichophyton isolates support our observation of high MIC-90 to terbinafine.11,21,22

Limitations of the study
A larger sample size involving different geographical locations would have given a more diverse view of the present dermatophyte epidemic in India. Genomic studies on the isolated species would have given us an insight into possible mutations. However, this was not possible in view of cost and infrastructure constraints. The study did not include in vivo correlation of responsiveness to drugs. The ideal model of antifungal drugs susceptibility should be inclusive of in vitro sensitivity, followed by measuring the level of the drug reached in stratum corneum and then observing clinical response of the drugs in vivo.

Conclusion
This study points to rising proportion of strains of Trichophyton mentagrophytes complex with higher MICs to antifungals like itraconazole and terbinafine. This study also confirms the epidemiological shift of dermatophytes in India toward an upsurge of T. mentagrophytes complex. It demonstrates the increasing proportion of granular form over downy form especially in T. mentagrophytes complex.

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Conflicts of interest
There are no conflicts of interest.

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