The Current Indian Epidemic of Dermatophytosis: A Study on Causative Agents and Sensitivity Patterns

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Abstract
Background: In the recent years, the frequency, severity, clinical characteristics, treatment response, and relapse rate of dermatophytosis have dramatically changed in India. Given the surge in dermatophytosis, we had undertaken a study to isolate and identify the common species causing dermatophyte infection and to know the in vitro efficacy of the common antifungals against them. Materials and Methods: A total of 103 new cases that were not on any treatment for the past 3 months were included. Skin scrapings were collected for direct microscopic examination and for fungal culture in Sabouraud 4% dextrose agar (SDA) with chloramphenicol and cycloheximide slant tubes, and dermatophyte test media. Fungi were identified on the basis of their macroscopic and microscopic features with the help of lactophenol cotton blue staining and urease test. Also, the drug sensitivity of the dermatophytes was tested with the common antifungals. Results: Of the 55 cases (53.4%) that were positive for dermatophytes in the culture, 29 showed possible contamination. Trichophyton was the predominant organism (49 cases) with T. verrucosum being the commonest species (26 cases), followed by T. rubrum (15 patients), and T. mentagrophytes (8 cases). All species of Trichophyton were found to be most sensitive to itraconazole amongst systemic antifungals and luliconazole amongst topical antifungals. Conclusion: This study concluded that the causative agent for the dermatophytosis was changing in India and in our subset, T. verrucosum caused the maximum number of infections. Itraconazole and luliconazole had the highest sensitivity amongst systemic and topical antifungals, respectively. It also showed that terbinafine had comparatively less sensitivity to most organisms.

Key Words: Antifungals, dermatophyte, itraconazole, tinea corporis, trichophyton verrucosum

Introduction
Over the past few years, it has been documented that dermatophyte infections have increased by many folds in India. Moreover, there is a change in the disease presentation, severity, treatment response, and relapse rate. Studies suggested that the emergence of Trichophyton mentagrophytes as the principal causative organism and high terbinafine resistance could be the cause of these changing patterns of the disease and response. However, the causes may even be more diverse, from the irrational use of antifungal drugs to topical steroid usage and also to the poor socioeconomic status of the population.

Though there are a few published studies from Karnataka, Sikkim, Delhi, and Himachal Pradesh, there are not much data available from eastern and Gangetic India where the disease is rampant. Moreover, there are not many studies determining the sensitivity pattern of the species of dermatophytes against various topical and systemic antifungal drugs.

Keeping all these factors in view, we had undertaken a study to isolate and identify the species causing dermatophyte infections in patients and to know the in vitro efficacy of the common antifungals against them.

Materials and Methods
It was a descriptive study conducted among the patients with dermatophytosis attending the dermatology outdoor of a tertiary healthcare center in eastern India. The study recruited patients over 6 months, from April 2018
to September 2018. A total of 103 patients were included in the study. Institutional ethical clearance was taken and all patients participated after signing informed consent form.

Patients of both gender presenting to the dermatology outdoor with the clinical diagnosis of dermatophytosis (tinea corporis, tinea faciei, and cruris) were included. Only new cases who were not under any treatment for the past 3 months were included. Patients below 12 years of age were excluded. Those with associated secondary pyoderma, systemic illness, or other infections were also excluded.

Skin scrapings were collected from the edge of the lesion with a sterile scalpel after the affected areas were decontaminated with 70% alcohol. The samples were taken in sterile containers. They were then sent to the department of microbiology and were used for fungal culture, species identification, and sensitivity determination.

**Direct microscopic examination**

All the samples were subjected to KOH preparation for direct microscopic examination. Three drops of 10% KOH plus 40% dimethyl sulphoxide (DMSO) were placed over a clean grease-free slide with skin scraping samples placed on that and covered with a coverslip. Slides were examined in light microscopy, and hyaline branching septate hyphae and arthrospores were identified.

**Fungal cultivation**

All samples were cultured on Sabouraud 4% dextrose agar (SDA) with chloramphenicol and cycloheximide slant tubes, and dermatophyte test media. Incubation was done aerobically at 37°C (for isolation of *T. verrucosum*) and at 28°C for other dermatophytes.

Cultures were observed, every alternate day, to check for the appearance of any fungal growth or production of any pigment over the reverse side of the slant. Lactophenol cotton blue staining was done of smears from culture-positive slants to detect presence of macro and microconidia. SDA slants were inspected for up to 4 weeks for growth.

**Species identification**

The culture was identified on the basis of their macro and microscopic features with lactophenol cotton blue staining and urease test. The diagnosis of the dermatophytic infection was confirmed by the positivity of the direct smear and culture.

**Drug sensitivity**

The cultured dermatophytes were subjected to drug sensitivity to the common antifungals. Sensitivity to itraconazole, fluconazole, terbinafine, griseofulvin, and voriconazole was tested. Among topical antifungals, sensitivity to clotrimazole, miconazole, ketoconazole, and luliconazole was determined.

All the samples were inoculated in SDA slant containing chloramphenicol and cycloheximide and incubated both at 37°C and at 28°C.

Antifungal drug susceptibility testing was done by the Clinical and Laboratory Standards Institute (CLSI) M38-A method. RPMI (Roswell Park Memorial Institute) broth was taken and inoculum of 0.4×10^5–5×10^6 CFU/mL was taken and *Trichophyton rubrum* (ATCC, MRL 666) was also used for quality control. The final concentration of antifungal drugs range was between 0.0625 to 256 mcg/mL for luliconazole, 0.0312 mcg/mL for fluconazole, 0.0312–32 mcg/mL for ketoconazole, 0.0312–256 mcg/mL for griseofulvin, and 0.0078 to 8 mcg/mL for itraconazole.

**Statistics**

Descriptive statistical analyses were done by SPSS for Windows (version 13.0, Chicago, IL).

**Results**

A total of 103 cases of clinical dermatophyte infection were included in this study. There were 66 (64%) female and 37 (36%) male patients. Sixty-three (61%) of them were young adults (18–40 years of age). Of these 103 patients, 89 had tinea corporis, 77 had tinea cruris, and 17 had tinea faciei. The majority (63, 61%) had an infection at more than one site. Samples were taken from the most representative and accessible sites as decided by the treating physician.

Some of the patients (37, 35.9%) had a history of similar illness in the past and the present episode was a relapse/recurrence. However, we have included only those cases who were not treated with any topical or systemic drug in the past 3 months. Some (52, 50.4%) had a family history of similar disease in the recent past.

Fungal elements could be detected in only 54 (52.4%) cases on direct microscopy. Eighty-four (81.55%) of the samples showed positive results on fungal culture. Out of these, 29 of the cases showed contamination, the most common contaminant being *Aspergillus species*. These cases were not included in further study and analysis. Some yeasts detected in a few samples were considered to be colonizing the skin.

Fifty-five cases (53.4%) were positive for dermatophytes on culture. All the patients who were positive in KOH showed positive culture and six more patients who were negative in KOH showed positive culture. The higher positivity in culture could be because of false negative results in direct KOH microscopy. *Trichophyton* was the predominant organism causing dermatophytosis in 49 cases. *T. verrucosum* was seen in 26 cases, followed by *T. rubrum* (15 patients) and *T. mentagrophytes* (8 cases) [Table 1].
**Results of drug sensitivity**

Dermatophyte sensitivity was tested to common antifungals [Table 2]. All three species of *Trichophyton* showed the highest sensitivity to itraconazole amongst oral antifungal drugs. Similarly, all three *Trichophyton* species showed highest sensitivity pattern to luliconazole amongst the topical antifungals.

The results showed that all three species of *Trichophyton*, namely, *T. mentagrophytes*, *T. verrucosum*, and *T. rubrum*, had a significantly high sensitivity to most oral antifungals, such as itraconazole, voriconazole, griseofulvin, and fluconazole. Systemic terbinafine did not show any significant benefit over other antifungals. Amongst the topical antifungals, luliconazole showed a higher sensitivity over others.

**Discussion**

Earlier studies from India have described *T. rubrum* as the commonest agent causing dermatophytosis.[6,8] *T. rubrum* was the most prevalent dermatophyte in Central and Northern Europe where it accounted for 80%–90% of the strains isolated.[10] A similar epidemiological situation was also seen in America.[10]

Jain et al. found 46% with *T. rubrum*, and 14% with *T. mentagrophytes* in Jaipur, Rajasthan in 2008.[11] Lakshmanan et al. demonstrated similar data from Tamil Nadu in 2011 with 79% with *T. rubrum*, and 14.5% with *T. mentagrophytes*. [12] *T. rubrum* predominated (38.2%), followed by *T. mentagrophytes* (27.2%) in a study from Goa published in 2014.[13]

However, there was a change in the dermatophyte pattern in India noticed during the last 5 years with a rising prevalence of *T. mentagrophytes* in many studies. In 2014, one study from Himachal Pradesh showed *T. mentagrophytes* causing about two-thirds of the infections.[7] Data from north Karnataka showed *T. mentagrophytes* to be the principal organism causing nearly half of the infections.[6] However, two studies found *T. mentagrophytes* and *T. rubrum* had near equal prevalence.[14,15] *T. mentagrophytes* was isolated as the most common species from Sikkim.[16] *T. interdigitale* was the most common isolate from Delhi accounting for 94% of cases.[5]

In our study, *T. verrucosum* was found to be the most common dermatophyte with 47% of cases positive for it. This was followed by *T. rubrum* (27%) and *T. mentagrophytes* (14%). *Epidermophyton* and *Microsporum* were relatively less common with 9% and 2% positivity, respectively.

The causes for the shift of causative organisms are not known but can be postulated to multiple factors, including steroid abuse, indiscriminate use of oral and topical antifungals, changing agent and host factors, and environmental changes.

One recent multicenter study in India has mentioned *T. mentagrophytes* as causing an overwhelming majority of infections (92.6%). This study did a genetic analysis of the fungal DNA. But, unlike our samples, they did not exclude treated cases of fungal infections and a majority of their cases were already treated with oral antifungals, topical antifungals, and topical steroids.[10] We believe, this could be a cause of a largely different data which is rather showing demography of steroid modified tinea, as 81.3% of their cases had prior applications of potent topical steroids.[10]

As discussed *T. mentagrophytes*, a zoophilic fungus has emerged to be replacing *T. rubrum* in most parts of India. However, there was not much data available from the eastern part of India. Our study revealed, for the first time, that *T. verrucosum* was the commonest organism in primary cases of tinea cruris and corporis which were not modified by treatment. Worldwide too, only few studies from the Middle East report *T. verrucosum* to be common, otherwise considered to be a rare organism.[17] We suggest further study with detail epidemiological factors including occupation, contact with animals and so on which can reveal the factors for this variation.

The second part of our study dealt with the drug sensitivity of the common dermatophytes, to both oral and topical antifungals. Though in the past 5 years, a number of studies tried to find out the prevalent causative organism in different parts of India, very little was done to find out the effectiveness of the available drugs in this changing scenario. It is a common finding

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**Table 1: Culture result (n=55)**

| Causative organism        | No of patients |
|---------------------------|----------------|
| *Trichophyton*            | 49 (89.1%)     |
| *T. verrucosum*           | 26 (53.1%)     |
| *T. rubrum*               | 15 (30.6%)     |
| *T. mentagrophytes*       | 8 (16.3%)      |
| *Epidermophyton*          | 5 (9.1%)       |
| *Microsporum*             | 1 (1.8%)       |

**Table 2: Sensitivity of dermatophytes to common antifungals**

|                | *T. verrucosum* | *T. rubrum* | *T. mentagrophytes* |
|----------------|-----------------|-------------|---------------------|
| Itraconazole   | 88%             | 85%         | 86%                 |
| Voriconazole   | 74%             | 65%         | 68%                 |
| Ketoconazole   | 44%             | 34%         | 48%                 |
| Luliconazole   | 85%             | 88%         | 82%                 |
| Terbinafine    | 26%             | 24%         | 26%                 |
| Miconazole     | 40%             | 42%         | 35%                 |
| Clotrimazole   | 35%             | 35%         | 35%                 |
| Fluconazole    | 44%             | 38%         | 42%                 |
| Griseofulvin   | 60%             | 65%         | 58%                 |
amongst dermatologists in this part of the world that the disease is becoming increasingly treatment unresponsive with frequent failures and relapses.[4]

In our study itraconazole was found to be the most efficacious drug showing 88%, 85%, and 86% sensitivity for T. verrucosum, T. rubrum, and T. mentagrophytes, respectively. The sensitivity of common dermatophytes to griseofulvin was also good, in the range of 58%–65%. Sensitivity to ketoconazole was between 34%–48%. However, terbinafine which was often considered to be the first-line antifungal for dermatophyte till recently, showed a very low sensitivity data of only 24%–26%.

Among the topical antifungals, luliconazole, clotrimazole, and miconazole were tested. Luliconazole was found to be the most effective with 82%–88% sensitivity. Clotrimazole was found to be least effective with 35% sensitivity while miconazole was found to be marginally better with 35%–42% sensitivity. Newer topical antifungals like amorolfine, eberconazole, and so on could not be tested.

The sensitivity of common dermatophytes to itraconazole is still quite high and therefore, the common perception that dermatophytes are not responding to itraconazole might either be due to improper dosing, poor compliance, socioeconomic and hygiene issues, or unsatisfactory quality of the brand used. An interesting finding in the current study points towards the very low sensitivity of terbinafine which was pointed out in some earlier studies as well.[5,18] In a study in Delhi the cases of such high-level resistance to terbinafine were attributed to harboring mutations in the squalene epoxidase (SQLE) gene.[5] In our study, griseofulvin showed relatively good sensitivity than fluconazole and terbinafine, which might be put to good use considering its low cost.

Limitations of the study
Samples were taken from only one center. As such, the generalizability of the findings is difficult. Larger sample size and molecular analysis were not found to be feasible on account of financial and technical constraints.

Conclusion
This study demonstrated that the causative agent for the epidemic of superficial dermatophytosis in India had undergone a marked change where the species had switched dramatically from T. rubrum to other species. In our study, the most common causative organism was T. verrucosum. This study also delineated higher sensitivity of itraconazole and luliconazole compared to other antifungals commonly in use in superficial dermatophytosis. It further demonstrated that voriconazole might not be a superior choice over itraconazole and that griseofulvin might be used in cases where itraconazole was contraindicated.

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Conflicts of interest
Sun Pharmaceutical Industries Ltd. has provided us with the active pharmaceutical ingredient (API) and the logistic support for the study.

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