

# To study the isolation of dermatophytes and its molecular characterization by using Multiplex PCR in tertiary care hospital

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**Abstract:** For the successful treatment of dermatophytoses, there is a need for accurate and rapid diagnostic methods. Dermatophytosis is caused by fungi from the Epidermophyton, Microsporum, and Trichophyton genera. Dermatophytes are keratinophilic (keratin-digesting) fungi that can cause disease. Human and animal pathogens are Microsporum and Trichophyton species, however Epidermophyton is an obligatory human pathogen. The present investigation was aimed to study the isolation of dermatophytes and its molecular characterization by using Multiplex PCR in tertiary care hospital. According to the current analysis using the microtube dilution technique, antifungal sensitivity testing for dermatophytic isolates revealed that terbinafine is a more sensitive agent for therapy and fluconazole is going towards resistance. Multiplex PCR is a useful method for identifying dermatophyte isolates since it is quick and easy to use. When compared to morphological and biochemical features, molecular approaches such as multiplex PCR improve the specificity of dermatophytosis laboratory diagnosis.

## INTRODUCTION

Dermatophytosis is a group of superficial fungal infections of keratinized structures such as the skin's stratum corneum, nails, and hair caused by dermatophytes. Like most other fungal diseases, the Kingdom Fungi comprises a diverse range of species. These organisms are particularly well adapted to infect skin and appendages because they can feed on keratin. Even in highly immunocompromised patients, dermatophytes seldom infiltrate deep tissues or cause systemic infections. Because of the likeness of its effect on wool garments to the shape of fungal skin lesions, the name tinea comes from the Roman word for clothes moth<sup>1,2,3</sup>. Dermatophytosis is caused by fungi from the Epidermophyton, Microsporum, and Trichophyton genera. Dermatophytes are keratinophilic (keratin-digesting) fungi that can cause disease. Human and animal pathogens are Microsporum and Trichophyton species, however Epidermophyton is an obligatory human pathogen.

Dermatophytic infections are found all throughout the world, however they are more common in tropical and subtropical nations. Because dermatophytes thrive in warm, humid environments, superficial fungal infections are rather frequent in tropical locations around the world<sup>3</sup>.

In clinical practise, a variety of antifungal medications are now available for the treatment of dermatophytosis, many of which are both safe and effective<sup>4</sup>. Drug-resistant dermatophytes that cause a variety of lesions, on the other hand, do not respond well to treatment<sup>5</sup>, implying that age, sex, hair involvement, nail growth, extent of nail involvement, peripheral vascular disease, fungal growth patterns, and the presence of dormant fungal arthrospores in the nail may all have an effect on antifungal treatment<sup>6,7</sup>. The capacity of an antifungal drug to suppress the fungal isolates is critical for successful treatment<sup>8</sup>. In vitro susceptibility testing using the microbroth dilution technique is useful in predicting this ability since it allows clinicians to determine the best treatment for their patients.

The Clinical and Laboratory Standards Institute (CLSI, previously the NCCLS) published standard protocol M38-A, which describes how to use the microbroth dilution technique to assess the susceptibility of dermatophytes to antifungal medications. Many researchers have presented a variety of approaches based on this procedure (M38-A), making it easier to compare results<sup>9</sup>. However, several test conditions have been analysed and shown to be reproducible and reliable<sup>10</sup>. Antifungal susceptibility testing of dermatophytes is done using broth microdilution techniques<sup>11,12,13</sup>.

Human infection by dermatophytes can be avoided by controlling animal disease. Antifungal agents should be used to treat infected animals, and suitable and particular disinfectants should be used on premises and fomites. When handling sick patients and animals, personal protective equipment such as hand gloves, masks, and full sleeve aprons should be worn. Identification of dermatophytic species is important not only for epidemiological reasons, but also for determining the types of isolates and sources of infection in a given area. Despite the availability of a large number of efficient antifungal medications, dermatophytic infection is a big concern all over the world, particularly in tropical nations like India.

Dermatophyte has survived various generations of therapy regimens, ranging from single dose antifungals to multiple dosage medications such as griseofulvin, tolnaftate, and imidazole, according to medical science. Some dermatophytes were formerly abundant but are now rare, possibly due to geographical limits. This suggests that economics, geography, species type, and environmental factors all play a role in dermatophytic survival and distribution. It is equally vital to study both anthropophilic and

zoophilic dermatophytes, which will become more prevalent in the near future as the number of immunocompromised diseases rises.

The present investigation was aimed to study the isolation of dermatophytes and its molecular characterization by using Multiplex PCR in tertiary care hospital.

## MATERIALS AND METHODS

The study comprised 600 clinically suspected dermatophytic infection cases from the outpatient department (OPD). To select probable dermatophytosis patients, a random selected sample method was applied. The respondents were then chosen using a systematic random sampling process.

The research was carried out in the Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore, MP, India, by collecting samples from OPD patients at the Department of Dermatology. After obtaining consent from each patient and their guardians, patients were chosen and participated in the study. Patients were evaluated, and samples of skin scrapings, hair plucking, and nail clippings were taken in accordance with conventional dermatophytes isolation and identification procedures<sup>14</sup>.

## RESULT

### Table 1. Distribution of cases according to the clinical types of tinea infection in relation to age

The samples were taken from patients ranging in age from 1 to 82 years old. The age group of 26-30 years had the highest number of specimens, with 235. (39.16 percent). Children aged 6-10 years had the second highest infection rate, with 86 (14.33 percent) and children aged 21-25 years had the third highest rate, with 79 (14.33 percent) (13.16 percent). The majority of the cases, 281 (46.84 percent), were tinea corporis, which was notable. Tinea capitis, which accounted for 75 percent of the cases, and tinea cruris, which accounted for 72 percent of the cases, were the next most common among patients of various ages.

| Clinical type                        | Age group of the patients in years |     |      |       |       |       |       |       |       |     | Total | %     |
|--------------------------------------|------------------------------------|-----|------|-------|-------|-------|-------|-------|-------|-----|-------|-------|
|                                      | <1                                 | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | 36-40 | >40 |       |       |
| tinea corporis                       | 5                                  | 0   | 10   | 14    | 28    | 56    | 134   | 19    | 8     | 7   | 281   | 46.84 |
| tinea capitis                        | 0                                  | 16  | 59   | 0     | 0     | 0     | 0     | 0     | 0     | 0   | 75    | 12.51 |
| tinea cruris                         | 0                                  | 0   | 0    | 4     | 7     | 11    | 29    | 7     | 11    | 3   | 72    | 12    |
| tinea pedis                          | 0                                  | 0   | 4    | 0     | 2     | 8     | 18    | 0     | 7     | 0   | 39    | 6.5   |
| tinea unguium                        | 0                                  | 0   | 0    | 0     | 0     | 4     | 22    | 3     | 4     | 3   | 36    | 6     |
| tinea manum                          | 0                                  | 0   | 0    | 0     | 3     | 4     | 8     | 3     | 0     | 0   | 18    | 3     |
| tinea faciale                        | 0                                  | 0   | 0    | 3     | 3     | 3     | 3     | 0     | 0     | 0   | 12    | 2     |
| Extensive tinea                      | 0                                  | 0   | 0    | 0     | 4     | 0     | 0     | 0     | 0     | 3   | 7     | 1.16  |
| tinea corporis/cruris                | 0                                  | 0   | 6    | 0     | 0     | 0     | 0     | 0     | 0     | 0   | 6     | 1     |
| tinea corporis/<br>barbae            | 0                                  | 0   | 0    | 8     | 6     | 0     | 17    | 6     | 7     | 0   | 44    | 7.33  |
| tinea<br>capitis/corporis            | 0                                  | 0   | 0    | 0     | 0     | 0     | 4     | 0     | 0     | 0   | 4     | 0.67  |
| tinea pedis/corporis                 | 0                                  | 0   | 0    | 0     | 0     | 0     | 0     | 0     | 3     | 0   | 3     | 0.5   |
| Tinea<br>unguium/corporis/<br>cruris | 0                                  | 0   | 0    | 0     | 0     | 0     | 0     | 0     | 0     | 3   | 3     | 0.5   |
| Total                                | 5                                  | 16  | 79   | 29    | 53    | 86    | 235   | 38    | 40    | 19  | 600   | 100   |

Table 2. Distribution of cases according to clinical type of disease in relation to dermatophytes

Only 353 (59.66%) of 600 clinically suspicious individuals had a positive culture. The isolates were mostly from three genera and eight species. Trichophyton mentagrophytes 109 (30.44%) and Trichophyton rubrum 84 (23.46%) were the most common isolates from tinea corporis. Trichophyton verrucosum 57 (15.92%) was the second most common dermatophyte.

The current study discovered that 45.81 percent of the patients had both tinea corporis and tinea capitis 15.52 percent, with tinea cruris accounting for 13.12 percent, tinea pedis for 7.54 percent, tinea unguium for 5.58 percent, tinea manum for 3.35 percent, and tinea barbae for 0.83 percent.

| Clinical type                 | Species of dermatophytes |                  |                     |                      |                     |                 |                     |                        | Total | %     |
|-------------------------------|--------------------------|------------------|---------------------|----------------------|---------------------|-----------------|---------------------|------------------------|-------|-------|
|                               | <i>T. mentagrophytes</i> | <i>T. rubrum</i> | <i>T. violaceum</i> | <i>T. verrucosum</i> | <i>E. floccosum</i> | <i>M. canis</i> | <i>T. tonsurans</i> | <i>T. schoenleinii</i> |       |       |
| tinea corporis                | 19                       | 39               | 34                  | 47                   | 3                   | 8               | 14                  | 0                      | 164   | 45.81 |
| tinea capitis                 | 20                       | 10               | 00                  | 0                    | 0                   | 0               | 17                  | 5                      | 52    | 15.52 |
| tinea cruris                  | 18                       | 12               | 7                   | 5                    | 2                   | 0               | 3                   | 0                      | 47    | 13.12 |
| tinea pedis                   | 19                       | 6                | 0                   | 0                    | 2                   | 0               | 0                   | 0                      | 27    | 7.54  |
| tinea unguium                 | 11                       | 8                | 0                   | 0                    | 3                   | 0               | 0                   | 0                      | 20    | 5.58  |
| tinea manum                   | 8                        | 4                | 0                   | 0                    | 0                   | 0               | 0                   | 0                      | 12    | 3.35  |
| tinea faciale                 | 0                        | 0                | 0                   | 2                    | 0                   | 0               | 0                   | 0                      | 2     | 0.55  |
| Extensive tinea               | 0                        | 0                | 0                   | 5                    | 0                   | 0               | 0                   | 0                      | 5     | 1.39  |
| tinea corporis/cruris         | 10                       | 2                | 0                   | 0                    | 3                   | 5               | 0                   | 0                      | 20    | 5.58  |
| tinea corporis/barbae         | 1                        | 2                | 0                   | 0                    | 0                   | 0               | 0                   | 0                      | 3     | 0.83  |
| tinea capitis/corporis        | 1                        | 0                | 0                   | 0                    | 0                   | 0               | 2                   | 0                      | 3     | 0.83  |
| tinea pedis/corporis          | 0                        | 1                | 0                   | 0                    | 0                   | 0               | 0                   | 0                      | 1     | 0.27  |
| Tinea unguium/corporis/cruris | 2                        | 0                | 0                   | 0                    | 0                   | 0               | 0                   | 0                      | 2     | 0.55  |
| Total                         | 109                      | 84               | 41                  | 57                   | 13                  | 13              | 36                  | 5                      | 358   | 100   |

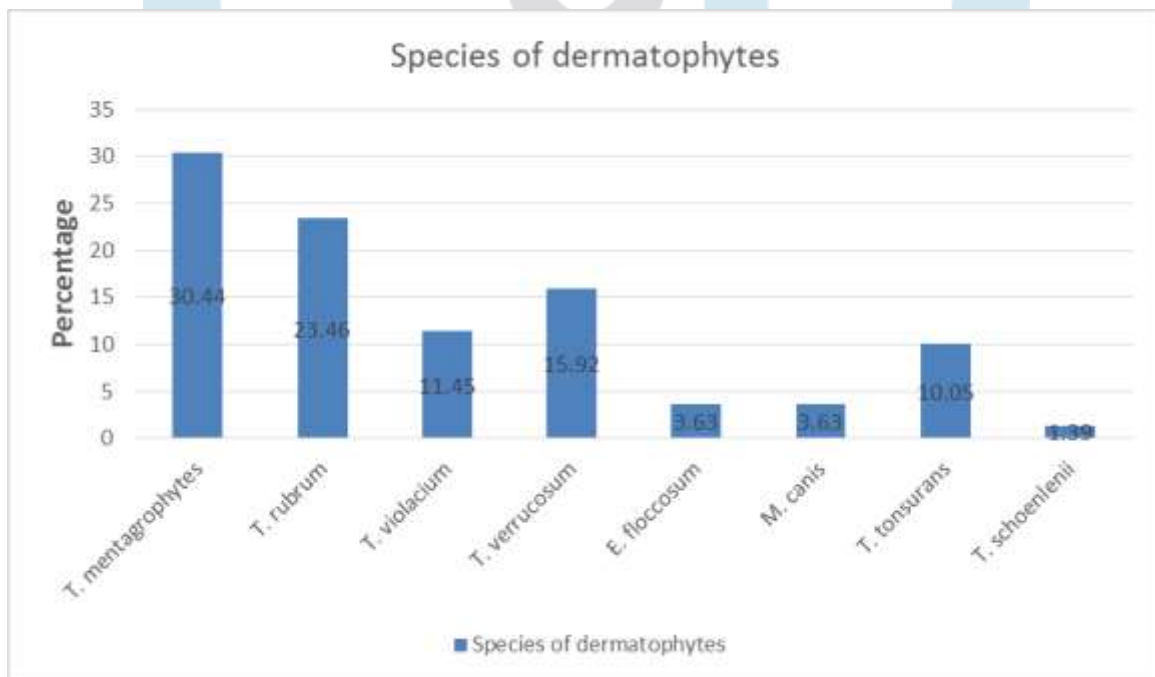
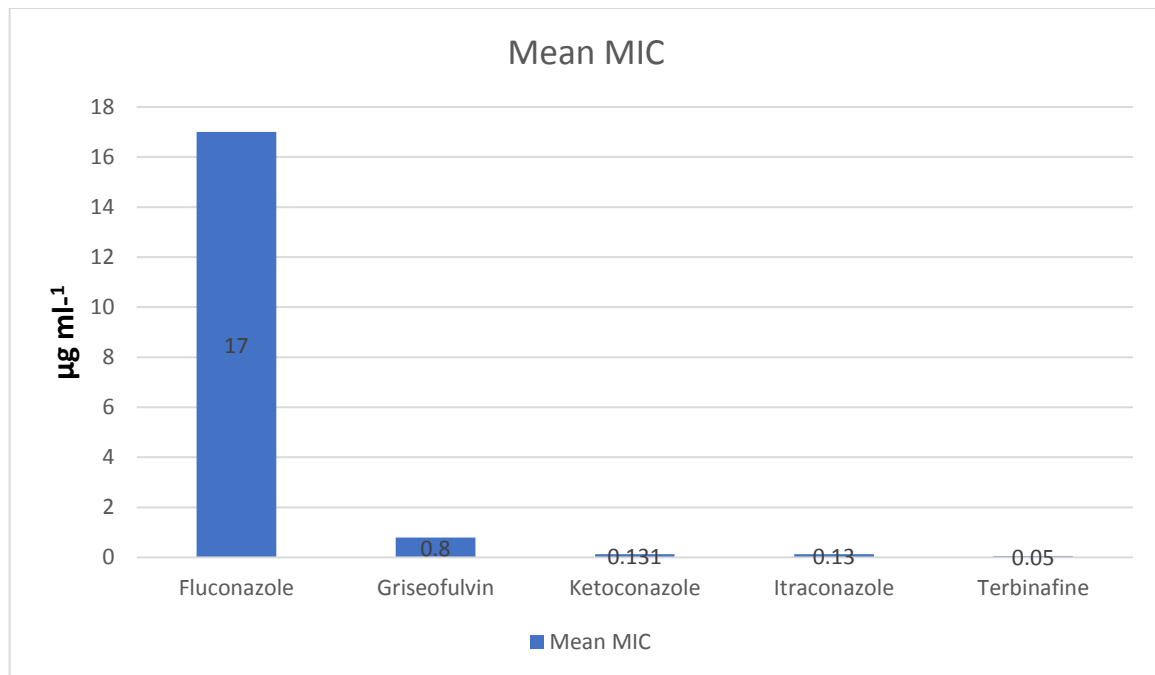


Figure 1. Distribution of cases according to clinical type of disease in relation to dermatophytes

Table 3. Mean MIC of antifungal agents by microtube dilution antifungal susceptibility test

The mean of the mean MICs for fluconazole, griseofulvin, ketoconazole, itraconazole, and terbinafine revealed that terbinafine (mean MIC<sub>50</sub>, 0.05 g ml<sup>-1</sup>; MIC<sub>90</sub>, 0.14 g ml<sup>-1</sup>) was 50% lower than the second most active antifungal agents like itraconazole and ketoconazole, which was statistically significant. Fluconazole was the least effective antifungal agent against dermatophytes as shown by microtube dilution technique.

| Antifungal agents | Mean MIC (µg ml <sup>-1</sup> ) |
|-------------------|---------------------------------|
| Fluconazole       | 17                              |
| Griseofulvin      | 0.8                             |
| Ketoconazole      | 0.131                           |
| Itraconazole      | 0.130                           |
| Terbinafine       | 0.05                            |



**Figure 2. Mean MIC of antifungal agents by microtube dilution antifungal susceptibility test**

## DISCUSSION

Out of 600 clinically suspected patient samples, 390 (65%) were skin scrapings, 120 (20%) were hair plucking's, and 90 (15%) were nail clippings, all of which were collected and processed for dermatophyte identification from various clinical kinds.

In a study of 600 clinically suspected individuals, males were more infected than females, with 348 (58.0 percent) infected compared to 252 females (42.0 percent).

The samples were taken from patients ranging in age from two months to 89 years old. The biggest number of specimens were found in the age group of 26-30 years, with 130 (21.67 percent) in the male group and 113 (18.84 percent) in the female group, which was statistically significant ( $P < 0.05$ ). The second greatest infection rates were identified in individuals aged 21–25 years old, namely in male group 43 (7.16 percent) and female group 44 (7.16 percent) (7.33 percent). In terms of gender, males were infected at a higher rate than females, with 348 (58.0 percent) infected compared to 252 females (42.0 percent).

The youngest patient in the study was a two-month-old boy, and one of the female patients was nine months old, while the oldest male patient was 89 years old. In both males and females, the highest rates of dermatophytic infection were reported between the ages of 26 and 30. This conclusion matched that of a prior study conducted by Shahindokht et al., (2009)<sup>15</sup> found that 40.5 percent of the patients were female and 59.5 percent were male. Jha et al., (2006)<sup>16</sup> found that the tinea capitis accounts for 4.6 percent of all dermatophytic infections, with 68.1 percent of cases occurring in children under the age of 11 years and a male to female ratio of 1:1.9 136. Singh et al., (2003)<sup>17</sup> investigated that the dermatophytic infection was found in 45.4 percent of patients aged 16 to 30 years and 25.8 percent of patients aged 31 to 45 years. 1.57:1 was the male-to-female ratio. Agrawalla et al., (2001)<sup>18</sup> reported an incidence of dermatophytosis. The most common age group in their study was 11-20 years, and the incidence of dermatophytosis was 4.54 percent with a M:F ratio of 2.5:1. In 68 percent of patients, there was just one clinical category, whereas 32 percent had two or more. Larone (1995)<sup>19</sup> which showed 52.0% of the infected males.

In total, 600 patients were included in this investigation. The majority of patients took home-made or over-the-counter medicine in partial doses for the first four weeks and only went to the doctor after the fifth or eighth week, 318 (53.0 percent) of infection.

The samples were taken from patients ranging in age from 1 to 82 years old. The maximum number of infections were discovered in 348 (58.0%) male patients with tinea corporis 133 (22.16%) and tinea cruris 98 (9.67%), and the highest infections were detected in 252 female patients with tinea corporis 148 (24.67%) and tinea capitis 29. (4.84 percent).

The samples were taken from patients ranging in age from 1 to 82 years old. The age group of 26-30 years had the highest number of specimens, with 235. (39.16 percent). Children aged 6-10 years had the second highest infection rate, with 86 (14.33 percent) and children aged 21-25 years had the third highest rate, with 79 (14.33 percent) (13.16 percent). The majority of the cases, 281 (46.84 percent), were tinea corporis, which was notable. Tinea capitis, which accounted for 75 percent of the cases, and tinea cruris, which accounted for 72 percent of the cases, were the next most common among patients of various ages. There was a male predominance in the gender-wise distribution of cases as compared to female patients.

The current finding is consistent with that of Bindu (2002)<sup>20</sup>, who also found male predominance (2.06: 1). Male populations may have a higher prevalence of tinea infection as a result of severe physical labour and long-term shoe wear, which may predispose them to fungal infection. Similar findings were reported by Singh et al., (2003)<sup>21</sup> from the western state of India, and Omar (2004)<sup>186</sup> from Egypt. Shahindokht et al., (2009)<sup>15</sup> from Tahrán, on the other hand, found a female majority in their study. The most common age group for tinea infection, according to Agrawalla et al. (2001)<sup>18</sup>, is 11 to 20 years old. In contrast to the reported finding in the current investigation, which showed that the youngest patient was 27 days old and the oldest was 77 years old, Bindu (2002)<sup>20</sup> found a similar result. In this study, 40.5 percent of those aged 26 to 30 had tinea infections, followed by 15 percent of those aged 21 to 25.



Only 353 (59.66%) of 600 clinically suspicious individuals had a positive culture. The isolates were mostly from three genera and eight species. *Trichophyton mentagrophytes* 109 (30.44%) and *Trichophyton rubrum* 84 (23.46%) were the most common isolates from tinea corporis. *Trichophyton verrucosum* 57 (15.92%) was the second most common dermatophyte. The current study discovered that 45.81 percent of the patients had both tinea corporis and tinea capitis 15.52 percent, with tinea cruris accounting for 13.12 percent, tinea pedis for 7.54 percent, tinea unguium for 5.58 percent, tinea manum for 3.35 percent, and tinea barbae for 0.83 percent.

Males had more tinea corporis instances in the current study than females, which is consistent with previous research. Male preponderance was revealed in studies by Bindu (2002)<sup>20</sup> and Singh et al. (2003)<sup>21</sup> from south and western India. It's possible that the increased occurrence of tinea corporis in male patients is due to the fact that males in India work in agriculture. Physical activity, as well as working in a hot, humid atmosphere, increases sweating, which may aid fungus parasitization. *T. rubrum* was identified as the major dermatophyte isolated in instances of tinea corporis in a study conducted in India by Singh et al. (2003)<sup>21</sup>. *T. mentagrophytes* was identified as the most common ring worm species by Agrawalla et al. (2001)<sup>18</sup> in Eastern Nepal. *T. verrucosum* was shown to be the most common causal agent of tinea corporis (28.2%), which is consistent with previous findings. Duke et al., (2004)<sup>22</sup> and Hay et al., (2004)<sup>23</sup>. were also achieved similar outcomes, *T. verrucosum* was isolated from all sites in individuals with widespread tinea infection (tinea corporis, tinea cruris, tinea manum, tinea unguium, and tinea pedis).

## CONCLUSION

Tinea corporis, tinea capitis, tinea cruris, tinea unguium, tinea pedis, tinea manum, tinea faciale, and widespread tinea were the clinical types of dermatophytic infections detected among the patients. *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton violaceum*, *Trichophyton verrucosum*, *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton tonsurans*, and *Trichophyton schoenleinii* were the most common species responsible for these infections.

PCR was used to test 138 cases of dermatophytes that were found to be culture positive. All phenotypically positive cultures were confirmed positive with 100 percent sensitivity and specificity using the PCR method.

According to the current analysis using the microtube dilution technique, antifungal sensitivity testing for dermatophytic isolates revealed that terbinafine is a more sensitive agent for therapy and fluconazole is going towards resistance.

Multiplex PCR with species-specific primers has been found to be a quick and accurate method for identifying dermatophytosis-causing strains from not only cultured colonies but also patient scales, and we expect it to be very useful in clinical practise.

Multiplex PCR is a useful method for identifying dermatophyte isolates since it is quick and easy to use. When compared to morphological and biochemical features, molecular approaches such as multiplex PCR improve the specificity of dermatophytosis laboratory diagnosis.

More research is needed to determine the major causes of infections as well as the infectivity of various dermatophytes in people and animals. Patients should be urged to dress in loose-fitting cotton or synthetic clothing that wick moisture away from the skin's surface.

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