



# Dermatophyte Culture at a Tertiary Healthcare Center in Nagaland: An observational study.

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## Introduction:

Over the last decade, there has been a substantial increase in reports of superficial dermatophytosis infections from across India.<sup>1</sup> Besides obtaining the status of a perennial disease, managing chronic dermatophytosis has placed a significant burden on clinical practice. Few studies have documented the prevalence of dermatophytosis in north-eastern states of India, but there is paucity of data from the state of Nagaland. This study assesses the common dermatophytes causing Dermatophytosis in patients presenting to a tertiary institute. Results from this report will provide data that will be used to plan and implement studies on the cause for chronicity and recurrence in the future.

## Methods:

An observational study was conducted on 641 clinically diagnosed superficial dermatophytosis patients, who visited the Department of Dermatology, between January 2018 and December 2021. Our institute is a tertiary healthcare institute in Dimapur, Nagaland. All new and recurrent cases of dermatophytosis visiting the Dermatology OPD were referred to the Department of Microbiology for sample collection and processing. After briefing the patient, the site was wiped with alcohol and skin scrapings from the active margins, or nail clippings or subungual debris was collected by trained laboratory personnel in a designated procedure room using a sterile surgical blade and transferred to a sterile and labelled plastic petri dish. The material was

processed inside a biosafety cabinet or work station where part of the scraping was subjected to direct microscopy with 10% KOH solution which was examined under 40X lens. The other part of the sample was transferred to Dermatophyte Test Medium (DTM) for culture. DTM is a selective culture medium for isolating and identifying dermatophytes that contains soya peptone and dextrose which support the growth of dermatophytes, and antibiotic supplement like cycloheximide, chlortetracycline and gentamicin for inhibiting the growth of other fungi and bacteria that may be present in the sample. Specimens were incubated at room temperature ( $25^{\circ}\text{C} \pm 2$ ) for 14 days and intermittently checked for macroscopic growth. A positive sample was then followed by LPCB (LactoPhenol Cotton Blue) mounting of the growth for microscopic examination and slide culture test, to speciate the dermatophytes.

Statistical Analysis: Using SPSS 21.0 software, Chi-Square test was used for finding the correlation between KOH and culture tests. The correlation between KOH and culture was expressed as percentages.

Approval from the **Institutional Review Board** was obtained.

### Results:

Out of 641 participants included in the study, 360 (56.16%) were male and 281 (43.84%) were females, M:F ratio was 1.28. Minimum age observed was 2 years, and the maximum age was 90 years. Most common age group affected was 21-30 years, comprising 31.20% (200) of the study participants, followed by 31-40 years with 25.18% (161).

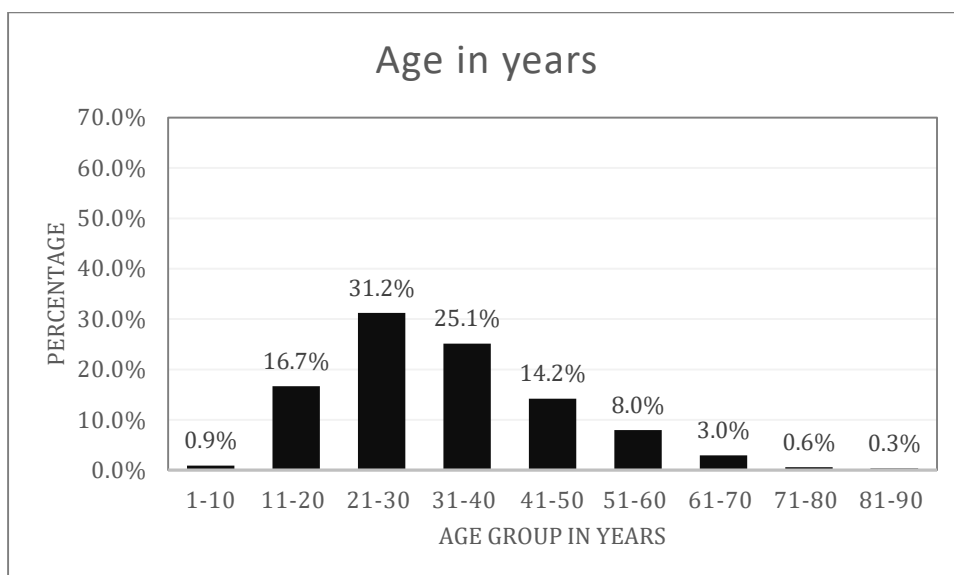


Fig.1. Bar graph showing the age distribution and prevalence of dermatophytosis.

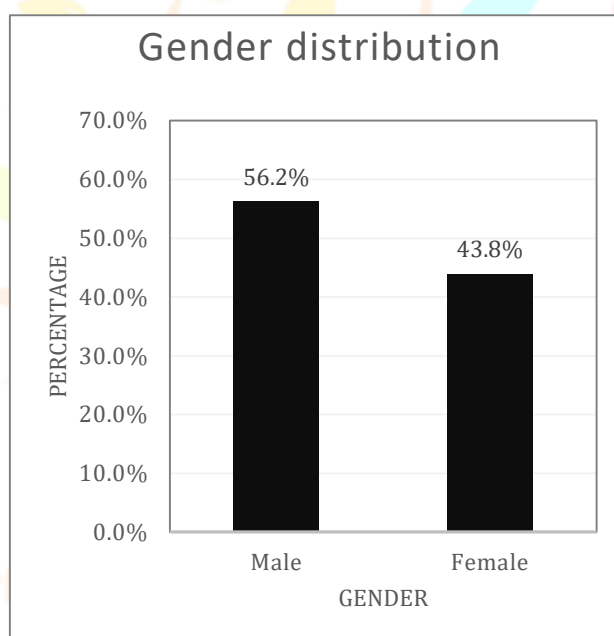


Fig.2. Bar graph showing sex distribution

KOH positivity rate was 80.34% (515/641) while culture positivity rate was 46.49% (298/641). Of the culture positive cases, *Trichophyton mentagrophytes* was the most common species isolated 61.07% (182/298), followed by *Trichophyton spp.* 30.20% (90/298) and *Trichophyton rubrum* 7.05% (21/298). Of the 641 participants, 539 (84.1%) were positive either for KOH or culture. However, 24 cases were culture positive but KOH negative.

<b>Dermatophyte species</b>	<b>n</b>	<b>%</b>
<i>T.mentagrophytes</i>	182	61.07
<i>T.spp</i>	90	30.2
<i>T.rubrum</i>	21	7.05
<i>E.floccosum</i>	2	0.67
<i>T.verrucosum</i>	1	0.36
<i>M.canis</i>	1	0.36
<i>M.tonsurans</i>	1	0.36

Table 1. Dermatophyte species isolated on culture

<b>Laboratory findings</b>	<b>n</b>	<b>%</b>
KOH + and Culture +	274	42.75
KOH + and Culture –	241	36.6
KOH – and Culture +	24	3.74
KOH – and Culture –	102	15.91
KOH + or Culture +	539	84.1

Table 2. KOH and culture correlation

Fig. 1. Off white flat, granular colony with central tuft of *Trichophyton mentagrophytes* on Dermatophyte Test Media after 10 days of incubation.

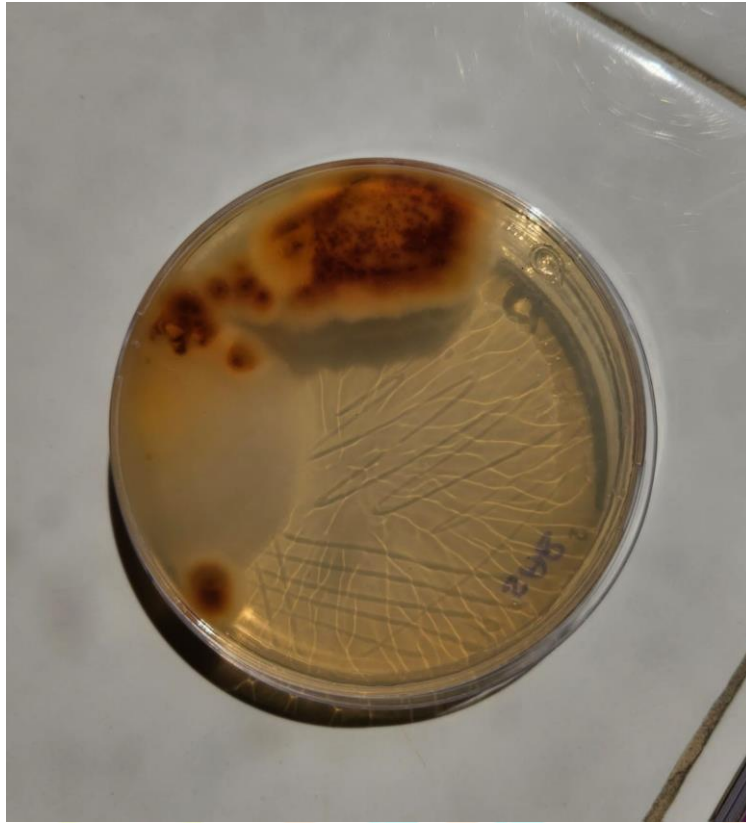


Fig. 2. Reverse yellow-brown pigmentation of *Trichophyton mentagrophytes* on culture.

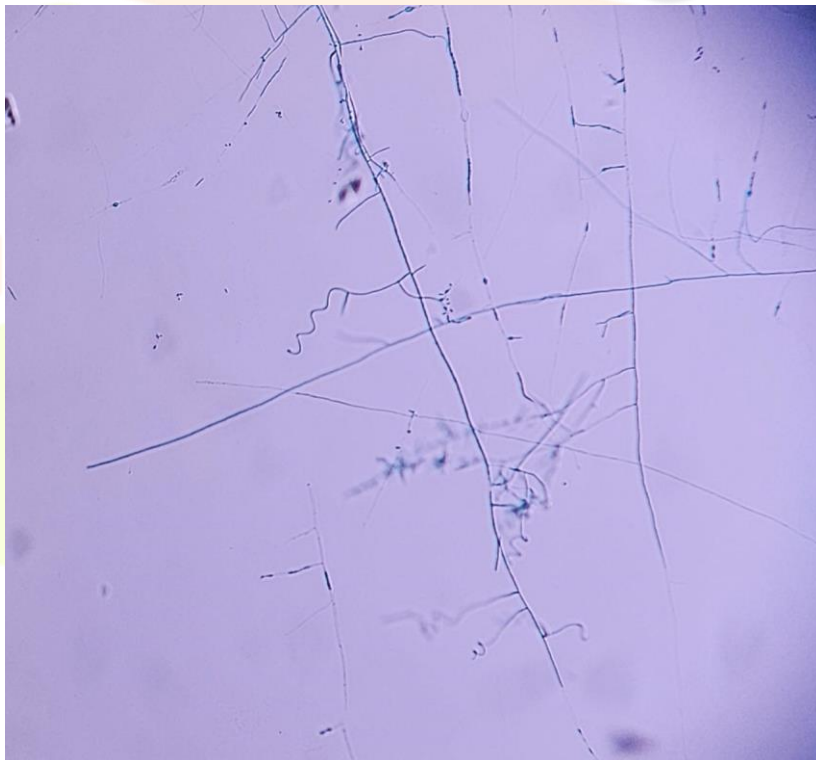


Fig. 3. LPCB mount of *Trichophyton mentagrophytes* showing spiral hyphae (40X)





Fig.4. LPCB mount of *Trichophyton rubrum* slide culture with clavate microconidia (40X).

#### Discussion:

This study aimed to assess common dermatophytes causing Dermatophytosis in patients presenting to a tertiary institute in Nagaland. The prevalence of dermatophytosis in India has increased exponentially over the decade, reported to be as high as 36.6%-78.4%.<sup>2</sup> A shift in the etiological agent is reported in literature from different parts of the country over the decade from *T. rubrum* to *T. mentagrophytes* that has led to anthropisation of the zoophilic species leading to easy transmission, longer survival on fomites and increased virulence.<sup>1,9</sup> In our study, *T. mentagrophytes* was the commonest organism isolated (table 1), followed by *Trichophyton spp* and *T. rubrum*. Other species we isolated were *Epidermophyton floccosum*, *Trichophyton verrucosum*, *Microsporum canis* and *Microsporum tonsurans*. There are very few studies from the north-eastern states of India with variable outcomes to compare with. But *T. rubrum* seems to still be the predominant species prevailing in other north eastern states like Assam and Meghalaya.<sup>2,3</sup> However, our findings are similar to Sharma R *et al* from Sikkim<sup>5</sup> Sahai *et al*<sup>6</sup> first reported the change in the etiological spectrum of dermatophytosis with 25% isolate comprising of *T. mentagrophytes* from Central India. Studies

from Odisha (Eastern India) also reported 77.50% to 79.22% of the isolates were *T. mentagrophytes*.<sup>7</sup> Nenoff *et al*<sup>9</sup> reported that 93.21% of their isolates were of *T. mentagrophytes* and 7.38% *T. rubrum*. They also identified a new genotype called *T. mentagrophytes* ITS type VIII implicated in drug resistant dermatophytosis and epidemic like situation of dermatophytosis. Saha *et al*<sup>10</sup> and Rajamohan R *et al*<sup>11</sup> reported *T. mentagrophytes* as the commonest isolate among chronic and steroid modified cases respectively. Pathania *et al* observed *T. mentagrophytes* as the predominant species in recalcitrant and recurrent dermatophytosis.<sup>12</sup> The recent studies done by Das D *et al*<sup>13</sup> and Saxena V *et al*<sup>14</sup> report *T. mentagrophytes* as the predominant species with 52.90% and 57.30% isolates respectively ; these are comparable with our estimates. Our study adds to the evidence from various studies,<sup>1,5-9,11-13,15,16</sup> that *T. mentagrophytes* is the commonest species causing Dermatophytosis currently, and this change in the spectrum may be attributable to the rise in chronic, recalcitrant dermatophytosis. Ours is a hospital-based study in a tertiary hospital, so only non-resolving or recurrent cases may come here. Most cases visiting our hospital have prior topical steroid use. Steroid availability over the counter (OTC) in Nagaland is rampant as there is no regulation in the private pharmacies with even smaller shops stocking steroid creams. Community based studies may give a better picture on the true patterns in the community.

Cutaneous Dermatophytosis affects all ages and sex.<sup>17</sup> In our study, participants of all age groups were included and we observed people in the second decade being primarily affected (figure 5) comparable with findings in studies done in Assam, Sikkim and Odisha.<sup>4,5,7,13</sup> The mean age among the study population was 33.18 years. Pathania *et al* observed most cases in the 3<sup>rd</sup> decade.<sup>12</sup> In all these studies including ours, males were more commonly affected than females (table 6) suggesting that the infection is more prevalent in the young males due to a lifestyle involving rigorous outdoor activities and manual labour that induces sweating creating a conducive environment for fungal infections. Studies conducted by Patro *et al*<sup>18</sup> and Sooriya S *et al*<sup>16</sup> show a reverse male female ratio indicating a rising prevalence in women too. The rising prevalence

convey women are equally susceptible to acquiring the infection possibly due to occlusive clothing habits worn by them, or more time being spent in hot or humid conditions of the kitchen or as a result of co-infection from other family members.

KOH positivity rate of 80.34% and a Culture positivity rate of 46.49% was observed. The low culture yield could be possibly due to prior or current use of antifungals or topical corticosteroids. Norhona *et al* observed a similar pattern in their study with a culture positivity rate of 40%.<sup>15</sup> Sharma R *et al*, Sahai *et al* and Jain S *et al* noted a culture positivity rate of 63.54%, 83% and 61.75% respectively.<sup>5,6,8</sup> In our study, KOH gave a better yield than culture. KOH is recommended as a point of care test that is simple, inexpensive, rapid and a good screening tool for diagnosing dermatophytosis. Although culture is considered the gold standard in diagnosing dermatophytosis, we observed KOH to be a better tool for screening and diagnosis, which is indicated by the high positivity rate of 80.34%. However, at instances KOH may not detect the fungus but may grow in culture as was seen in 24 cases in our study. A positive culture yield on a KOH negative sample could be due either to the presence of viable but low levels of fungal elements undetectable on KOH microscopy, or technical incompetence of the laboratory personnel in detecting the hyphae on microscopy. A negative culture report may be due also to the presence of dead or non-viable fungal elements, or due to the presence of inhibitory substances that can suppress the growth of the fungi. There is also the possibility of non-dermatophyte fungi being detected in KOH examination, which cannot grow in DTM, leading to a negative culture report. Hence, interpreting KOH results needs clinical correlation and acumen.

**Limitations:** Clinical profiling was not done for our patients, which could have also provided a picture on the spectrum of chronic and recalcitrant dermatophytosis in this region.

**Conclusion:** Chronic, recurrent and recalcitrant dermatophytosis continues to be a prevailing problem in India<sup>19</sup> that poses a great therapeutic challenge to the dermatologists. This could be as a result of the change in the etiological shift of the dermatophyte<sup>1,5,6,8,9,11-13,15,16</sup> due to various external, internal, immunological



mechanisms and virulence of the organism. Over and above these factors, injudicious use of topical corticosteroids has contributed to the re-emergence of the organism.<sup>9,11,20</sup> Our study also shows *Trichophyton mentagrophytes* as the predominant species in a tertiary healthcare setting in Nagaland, where misuse of topical corticosteroids amongst dermatophytosis patients is rampant. This change in the trend of species prevalence may be attributable to the spectrum of chronic dermatophytosis<sup>9,11,20</sup> in this region which requires further research.

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