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Phenotypic Identification, Diagnosis and Isolation of Dermatophytes from Kanpur Region, India: A Cross-Sectional Case Study

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A B S T R A C T

Dermatophytosis is a superficial clinical condition in which patients experience verities of manifestations in the skin. This disease has a high prevalence in developing countries and affects millions of people worldwide. Geographical variations, species similarities, and poor diagnosis systems are the key barriers to their therapeutic management. In this case study, we were intended to study the diagnosis and frequency distribution of dermatophytes in the Kanpur region, India. Aim and Objective: Phenotypic Identification, Diagnosis, and Isolation of Dermatophyte by using macroscopic as well as direct KOH microscopic investigation followed by culture examination. This was a cross sectional case study carried out in the Department of Microbiology, RMCH&RC, for the period of about 1 year from February 2017 to May 2018. The Phenotypic identification, diagnosis, and isolation of dermatophyte by using macroscopic as well as direct KOH microscopic investigation followed by culture examination were done. A total of 300 suspected individuals were included in this study to evaluate the causative agents of dermatophytosis. Out 300 suspected individuals, 144 patients were clinically diagnosed and mycologically confirmed as dermatophytes. We have isolated different species of dermatophytes of which *T. mentagrophyte* (56.31%) and *T. rubrum* (23.30%) were the most frequently observed contagious agents for humans. Further, we noted that the frequency of *T. mentagrophyte* (33.1%) and *T. rubrum* (14.56%) was higher in younger individuals as compared to children and the older population. Subsequently, we observed that *T. mentagrophyte* (54.37%) and *T. rubrum* (20.39%) were the most commonly isolated species from the skin than hair and nail. The prevalence of *T. mentagrophyte* and *T. rubrum* were notably higher in the Kanpur region of India. Therefore, the development of the novel, precise and rapid diagnostic tool is recommended to lead to the management and cure of dermatophytosis. Based on the above background, in this study, we have evaluated the phenotypic identification, diagnosis, and isolation of dermatophytes from suspected cases.

Introduction

Superficial fungal infection is a common and universal skin problem affecting millions of people worldwide (Pierard *et al.*, 1996; Bongomin *et al.*, 2017). The prevalence (estimated as 20-25%) and the incidence are continually increasing and correlated in respect of their molecular/genetic variation according to ethnicities (Zhan and Liu, 2017).

The principal etiologic agent responsible for superficial fungal infection is dermatophytes contributing 3%-4% of dermatological (Aly, 1994; Havlickova *et al.*, 2008). Although, there is a very faint chance to die due to this disease. It is a cutaneous infection interfering with the quality of life via modulating social stigma and upsetting routine activities (Evans & Gentles, 1985). However, the frequency of this disease is prominently higher in developing countries due to the high population index, socioeconomic burden, poor health status, and inadequate hygiene (Moto *et al.*, 2015). The routine diagnosis method for phenotypic identification is mostly accomplished through the macroscopic method to study colony morphology (Theel *et al.*, 2011; Nenoff *et al.*, 2014b). In the current scenario, the DNA sequencing approach is considered a "gold standard" method for species identification, however, due to expensive management; this is limited in most clinical settings (Nenoff *et al.*, 2019).

Materials and Methods

This hospital-based study was conducted between February 2017 to May 2018 at the Department of Microbiology, RMCH & RC Mandhana, Kanpur. A total of 300 clinically suspected cases of dermatophytosis were included in this investigation irrespective of their age, gender, and ethnicity.

Identification and Isolation of the Sample

The clinical samples were collected from skin, hair, and nail. Suspected lesion areas were sterilized and cleaned with 70% ethanol to disinfect the lesions and remove any dirt. Skin samples were collected from epidermal scales by scraping from close to the advancing edges of the lesions using the blunt edge of a sterile surgical blade onto sterile glass slides.

Hair samples were collected from the basal root portion by pricking the hair with sterile forceps. Nail samples were collected by scraping across the inflamed area of the lesions. After sample collection specimens were placed in sterile Petri dishes with proper labeling of patients' details. After that suspected cases were diagnosed by using microscopic and culture examination methods.

Phenotypic Identification and Diagnosis

All the suspected specimens were divided into two portions. The first portion of the specimens was examined using microscopy technique using 20% potassium hydroxide (KOH) with 40% dimethyl sulfoxide (DMSO) mount.

Specimens were thoroughly examined under 10X and 40X magnification using the binocular microscope to validate the presence of hyphae and/or arthroconidia. The second portion of specimens was cultured on Sabouraud's dextrose agar (SDA) medium containing chloramphenicol (0.05%) with and cycloheximide (0.5%) and Dermatophyte test medium (DTM) followed by incubating at 25°C for 2 to 4 weeks.

For species identification, culture positive for dermatophytes were examined macroscopically (color of the surface and reverse, topography, and texture) and microscopically (small unicellular microconidia and larger septate macroconidia).

In case of absence of any growth after 4 week then the culture was treated as negative for dermatophytes. All the required chemicals and culture medium were purchased from Himedia, India. The mycological identification was done by direct macroscopic and microscopic examination of the culture isolates.

Statistical analysis

Data were analyzed using Excel and expressed as frequency and percentage.

Results and Discussion

The clinical specimen of various origins (Skin, Hair, and Nail) was collected and evaluated to compare the classical diagnostic tools including direct microscopic observation and culture-based method followed by isolation of fungus.

We have clinically investigated a total of 300 suspected individuals (Table 1). Out of which, 144 (48%) individuals were found positive for dermatophytosis, whereas 156 (52%) individuals were recorded negative for the causative agent of dermatophytosis.

We have also distributed study samples based on clinical investigation methods including KOH microscopic and culture. According to that, 86 (28.67%) cases were found positive and 139 (46.33%) were recorded negative for both the methods. However, 58 (19.33%) cases were negative in the culture method but positive in direct microscopic examination. In respect to the culture-based method, 17 (5.67%) cases were positive for dermatophytosis but noted negative by microscopic examination. Collectively, our result indicated that the frequency of the direct microscopic method for the diagnosis of dermatophytosis was comparatively higher vs. the culture method.

Different clinically diagnosed and mycologically confirmed species of dermatophytes were represented in Table 3.

According to our finding, *Trichophyton mentagrophyte* (56.31%) and *Trichophyton rubrum* (23.30%) were the most commonly reported dermatophyte species followed by other species. Further, we represented the distribution of dermatophytes obtained from an isolate from different clinical specimens including Skin, Nail, and Hair (Table 3).

Our study demonstrated that out of 103 positive cases, 96 (93.20%) cases belonged to skin samples and 7 (6.80%) were isolated from the nail. However, no positive cases were isolated from the hair sample. We have also distributed dermatophytes based on species. Based on this, *T. mentagrophyte* (54.37%) and *T. rubrum* (20.39%) were the most commonly isolated dermatophyte species from the skin sample followed by other species. The frequency of species isolated from hair and nail was lower than species isolates from the skin.

Gender and age-wise distribution of dermatophytes were demonstrated in Tables 4 and 5 respectively.

Our finding indicated that the frequency of dermatophyte species was higher in males (56.31%) as compared to females (23.30%). Further, we noted that *T. mentagrophyte* (50.49%) and *T. rubum* (18.45%) was highly reported agent in male than female followed by other species. The patient's positive for dermatophytes belongs to different age groups. Of which, patients with age group between 15 to 30 years were found more susceptible to dermatophytosis infection than other age groups mentioned in table 5. Further, we also noted that the prevalence of *T. mentagrophyte* (33.1%) and *T. rubum* (14.56%) was higher in the age group between 15 to 30 years than in another age group in respect of other species.

Dermatophytosis is a major public health concern impacting tropical and subtropical countries including India. It is one of the contagious dermatophytic infections affecting the keratin-containing part of the body.

Table.1 Comparative analysis methods used for diagnosis of Dermatophytosis (n=300)

S. No.	Direct microscopy	Total n (%)	Culture	
			Positive n (%)	Negative n (%)
1	Positive n (%)	144 (48.0%)	86(28.67%)	*58(19.33%)
2	Negative n (%)	156 (52.0%)	17(5.670%)	139(46.33%)
3	Total	300	103(34.33%)	197(65.67%)

Table.2 Distribution of dermatophytes according on the basis of species (n=103)

S. No.	Dermatophytes	Total number (n)	Percentage (%)
1	*T.mentagrophyte*	58	**56.31%**
2	*T. rubrum*	24	**23.30%**
3	*T.tonsurans*	13	**12.62%**
4	*T. interdigitale*	03	**2.91%**
5	*T. terrestre*	02	**1.94%**
6	*E. floccosum*	02	**1.94%**
7	*M. audouinii*	01	**0.97%**
8	**Total**	**103**	**100%**

Table.3 Distribution of positive cases on the basis of clinical specimens (n=103)

S. No.	Dermatophytes	Nail (%)		Hair (%)		Skin (%)		Total Number	Percentage (%)
1	*T.mentagrophyte*	02	1.94%	0	0%	56	54.37%	58	**56.31%**
2	*T. rubrum*	03	2.91%	0	0%	21	20.39%	24	**23.30%**
3	*T.tonsurans*	00	0%	0	0%	13	12.62%	13	**12.62%**
4	*T. interdigitale*	00	0%	0	0%	03	2.91%	03	**2.91%**
5	*T. terrestre*	00	0%	0	0	02	1.94%	02	**1.94%**
6	*E. floccosum*	02	1.94%	0	0%	00	0%	02	**1.94%**
7	*M. audouinii*	00	0%	0	0%	01	0.97%	01	**0.97%**
8	**Total**	**07**	**6.80%**	**00**	**%**	**96**	**93.20%**	**103**	**100%**

Table.4 Gender wise distribution of dermatophytes (n=103)

S. No.	Dermatophytes	Male	Percentage (%)	Female	Percentage (%)	Total number	Percentage (%)
1	*T.mentagrophyte*	52	50.49%	6	5.83%	58	**56.31%**
2	*T. rubrum*	19	18.45%	5	4.85%	24	**23.30%**
3	*T. tonsurans*	12	11.65%	1	0.97%	13	**12.62%**
4	*T. interdigitale*	3	2.91%	0	0%	03	**2.91%**
5	*T. terrestre*	2	1.94%	0	0%	02	**1.94%**
6	*E. floccosum*	2	1.94%	0	0%	02	**1.94%**
7	*M. audouinii*	1	0.97%	0	0%	01	**0.97%**
8	**Total**	**91**	**88.35%**	**12**	**11.65%**	**103**	**100%**

Table.5 Age wise distribution of Dermatophytes (n=103)

S. No.	Dermatophytes	1-15 years (%)		15-30 years (%)		30-45 years (%)		45-60 years (%)		Total Number	Percentage (%)
1	*T.mentagrophyte*	1	0.97%	34	33.1%	18	17.48%	5	4.85%	58	**56.31%**
2	*T. rubrum*	1	0.97%	15	14.56%	4	3.88%	4	3.88%	24	**23.30%**
3	*T.tonsurans*	1	0.97%	8	7.77%	2	1.94%	2	1.94%	13	**12.62%**
4	*T. interdigitale*	0	0%	2	1.94%	1	0.97%	0	0 %	3	**2.91%**
5	*T. terrestre*	1	0.97%	1	0.97%	0	0%	0	0 %	2	**1.94%**
6	*E. floccosum*	0	0%	2	1.94%	0	0%	0	0%	2	**1.94%**
7	*M. audouinii*	0	0%	0	0%	1	0.97%	0	0%	1	**0.97%**
8	**Total**	**3**	**2.91%**	**62**	**60.19%**	**26**	**25.24%**	**12**	**11.65%**	**103**	**100 %**

Fig.1-5 Showing the phenotypic appearance of dermatophytes

Fig.1 *Tinea corporis* **Fig.2** *Tinea Capitis* **Fig.3** *Tinea unjuium*

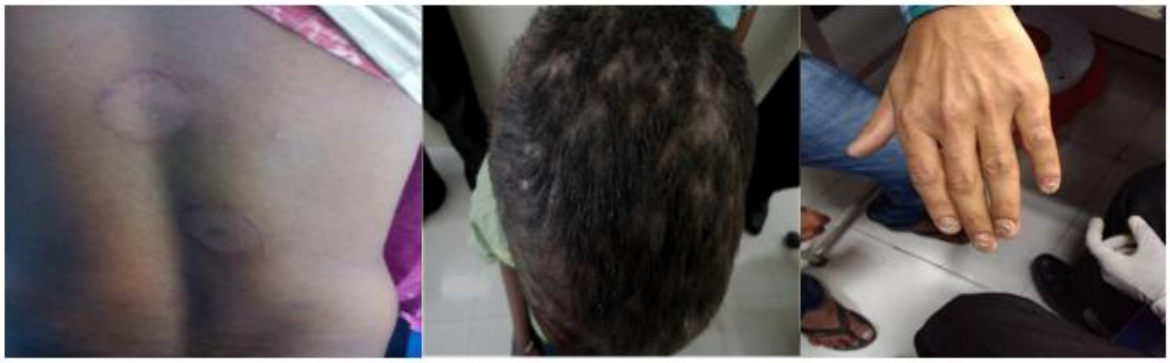

Fig.4 *Tinea mannam* **Fig.5** *Tinea pedis*

Fig.6 Microscopy KOH preparation

Fig.7 Slide culture technique

The most widely used conventional method for diagnosis, phenotypic study, diagnosis, and isolation of dermatophytes are microscopic observation followed by macroscopic examination and culture method to implement suitable treatment and prevention measures (Barry & Hainer, 2003). Studies demonstrated that the prevalence and incidence of *T. mentagrophyte* and *T. rubum* were higher in many ethnicities worldwide (Lee *et al.*, 2015). In our finding, we also found data in a similar trend as the above finding. However, the reason behind this is still unresolved and needs large-scale studies to uncover the science behind this. Our study revealed that direct microscopy is a more sensitive, specific, cheaper, and faster method for laboratory diagnosis of dermatophytosis followed by culture examination. Further, we demonstrated that the species of genus *trichophyton* namely *t. mentagrophyte* has been found as the most common dermatophyte followed by *T. rubum*. Our finding is coinciding with other previous studies (Kannan *et al.*, 2006). However, some authors reported the opposite trend i.e. the frequency of *T. rubum* was significantly higher vs. *T. mentagrophyte* in findings. It might be due to variation in species possibly due to climate change, migration, etc. Further, we reported that male individuals were more prone to dermatophyte infection than females. Notably, we also observed that the young age group was most widely affected by dermatophytes than children and the older age group population. This may be correlated with the occupation hazards related to their nature of work and the frequent interaction with different peoples of the society and exposure to the environment. The lower incidence in females may be due to the non-reporting of the female patient to the hospitals due to the prevailing social stigma in the rural population in India. This observation was supported by some of the earlier reports (Suman & Beena, 2003). We observed the dermatophytosis is common in the farmer, industrial section, and those people who work in soil and water. Most of the cases are found in an individual with exiting clinical conditions like diabetes, leprosy, and immune-compromised condition. The Microscopic examination is most precisely able to diagnose causative agents of dermatophytosis as compared to the culture technique. Thus, it may be a good strategy to utilize the microscopy method for the early diagnosis of patients positive for dermatophytosis to improve the treatment outcomes. Further, our findings state that dermatophytosis is the most common skin disease in the rural population in and around Kanpur, India. In addition, the discovery of a high throughput diagnosis technique is highly recommended to overcome the time to improve specificity and early treatment.

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Conflicts of interest

The authors declare that there is no potential conflict of interest associated with this study.

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