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Original Article

Predominance of *Trichophyton Mentagrophytes* from Superficial Mycoses Cases in Rural Population in North India: A Hospital Based Study

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ABSTRACT

Aim: A study was planned to know the prevalence of fungal isolates from superficial mycoses cases from rural population attending a tertiary care hospital in Lucknow. **Materials and methods:** A prospective study was conducted at a tertiary care hospital in Lucknow, Uttar Pradesh. Laboratory data of 75 clinically suspected cases of superficial mycoses attending Rural Health Training Centre of the institute were enrolled in the study. **Results:** Out of total 75 cases, majority 54 (72%) were male and 21 (28%) were female, most common age group affected was 31-40 years (28%), followed by 21-30 (21.3%), males were predominant (72%) and most common clinical presentation was tinea corporis in 38.7% cases, followed by T. cruris (22.8%), T. pedis (18.6%), T.capitis and T. barbae (4% each). On KOH examination, 52 (69.4%) samples were positive, while 23 (30.6%) cases were negative, 59 (78.6%) samples turned out to be culture positive. Most common etiological agent was *T. mentagrophytes* in 16 (27.1%) cases, followed by *T. rubrum* in 10 (16.9%), *T. tonsurans* and *M. audouinii* in 5(8.5%) cases each, *T. interdigitale* and *T. schoenleinii* in 2(3.8%) cases each. Eleven samples showed growth of contaminants while *Candida* spp.in 2(3.8%) and *Malassezia* spp. in 3(5.8%) and non dermatophyte was isolated from only 1(1.9%) case. **Conclusion:** In the current era of urbanization and globalization frequency of dermatophytes is changing from one region to other. Therefore, in a vast country like India, it is prudent for medical mycologists to remain update to diagnose uncommon species of dermatophytes for proper management of superficial mycoses cases

Key Words: *superficial mycoses, rural population, North India*

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INTRODUCTION

Superficial mycoses are fungal infections of skin, and its appendages (hairs and nails) caused by dermatophytes, yeasts and non-dermatophyte moulds. It includes dermatophytosis, dermatomycosis, pityriasis versicolor, white and black piedra.[1]

According to World Health Organization (WHO) superficial mycoses are believed to affect **20%** to **25%** of the world's population and the incidence continues to increase.[2]

Dermatophyte infections are one of the earliest known infections of mankind and are very common throughout the world**.** Although dermatophytosis do not produce mortality, it does cause morbidity and poses a major public health problem, especially in tropical countries like India due to the hot and humid climate.[3] Three genera of dermatophytes namely *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton floccosum* are causative agents of dermatophytosis. However, non-dermatophytic moulds (NDM) and Candida spp. are also responsible for causation of infections in nails and skin known as dermatomycosis.[4] In India various studies have been conducted in different parts of the country having varied climatic conditions and have reported different percentage of prevalence. There is scarcity of studies on fungal isolates in superficial mycoses cases from rural population in India, therefore this study was planned to know the spectrum of fungal isolates from superficial mycoses cases from rural population attending a tertiary care hospital in Lucknow.

Materials and Methods

A prospective study was conducted in the department of Microbiology, Integral Institute of Medical Science & Research, Lucknow, UP. The study population comprised of 75 clinically suspected cases of superficial mycoses attending RHTC of our medical college. Written informed consent was taken from every adult patients and legal guardians in case of pediatric patients. Patients not willing to give consent and patients with history of receiving antifungal treatment within one month were excluded from the study. The study was approved by the Institutional Review Committee (IRC) & the Ethical Review Committee (ERC). Statistical analysis of the data was done by using Chi square or Fischer test and p value of <0.05 was taken as significant.

Skin scrapping or hairs or nail bed scrapings were taken with the help of a sterile scalpel blade, from the affected region after cleaning the site with methylated spirit. Specimens were collected in a clean black paper for transportation to the laboratory for further processing.

All specimens were divided into two parts for direct microscopy and culture inoculation. Direct microscopy was done with the help of KOH mount by using 10% KOH solution for skin and hair specimens and 40% KOH for nail specimens. After keeping mounts for 1-2 hours for clearing, they were examined under low and high power objectives for the presence of filamentous, septate, branching hyphae with or without spores. In case of hairs, arrangement of spores was also noted and reported as endothrix or ectothrix. Negative specimens on first examination were further incubated overnight at 37˚C for clearing, before reporting them negative.

Pityriasis versicolor infections were diagnosed by the presence of characteristic 'meat balls and spaghetti' appearance in KOH mount and culture was not put for such cases.

All the samples collected were inoculated in two sets of test tubes containing Sabouraud dextrose agar with chloromycetin, Sabouraud dextrose agar with chloromycetin and actidione. The fungal cultures was identified by colony morphology, rate of growth and pigment production. Lactophenol cotton blue mount was done from the small bit of colony taken on clean glass slide and teased out using two teasinig needles, to detect the presence of macroconidia, microconidia, chlamydospore and special hyphal structures. Confirmatory identification of the species was done by slide culture technique. Other biochemical tests used were urease test, hair perforation test and rice grain test.[5]

Results

A total of 75 patients were enrolled in the study and none of them had any systemic disease. 54 (72%) were male patients and 21 (28%) were from female patients with the male female ratio of 2.5:1, this difference was found to be statistically significant (p<.ooo1). (Table 1)

Table 1: Gender distribution of clinical types

Clinical types	Cases n (%)	Male n (%)	Female n (%)	M/F ratio	P value
Tinea corporis	29 (38.7)	17(22.8)	12(16)	1.5:1	0.4
Tinea cruris	17 (22.8)	14(18.6)	3(4)	4.6:1	**0.03**
Tinea pedis	14 (18.6)	11(14.6)	3(4)	3.6:1	0.1
Tinea capitis	3 (4)	3(4)	0	N.A	N.A.
Tinea barbae	3 (4)	3(4)	0	N.A	N.A.
Tinea unguium	2 (2.6)	2(2.6)	0	N.A	N.A.
Tinea manuum	2 (2.6)	1(1.3)	1(1.3)	1:1	1.0
Tinea faciei	1(1.3)	0	1(1.3)	N.A	N.A.
Pityriasis versicolor	3 (4)	2(2.6)	1(1.3)	2:1	1.0
Piedra	1 (1.3)	1(1.3)	0	N.A	N.A.
Total n (%)	75 (100.0)	54(72)	21(28)	2.5:1	**0.0017**

N.A- not applicable due to low sample size, (p≤0.05=significant), (*=insignificant)

On analyzing age distribution of the total 75 suspected cases of superficial mycoses, T. corporis 21(28%) was the most clinical manifestation found in the age group of 31-40 years followed by 8 (10.6%) in 21-30 years, 7(9.3%) in 11-20 years, 4(5.3%) in >51 years, 3(4%) in 41-50 years while no cases in 0-10 years of age group.
T. cruris 6 (8%) was the most common clinical type found in the age group of 31-40 years followed by 4(5.3%) in 41-50 years, 3 (4%) in >51 years and least in age group of 11-20 years and 21-30 years with no cases in 0-10 years of age group.

T. pedis 6(8%) was most common manifestation in the age group of 31-40 years followed by 3(4%) in 21-30 years, 2(2.6%) in 31-50 and >51 years and least in age group of 11-20 years 1(1.3%).**(Table 2)**

Table 2: Age distribution of superficial mycoses cases

Clinical types	Age groups in years						
	Cases n (%)	0-10 n (%)	11-20 n (%)	21-30 n (%)	31-40 n (%)	41-50 n (%)	>51 n (%)
Tinea corporis	29 (38.7)	0 (00)	7(9.3)	8(10.6)	7(9.3)	3(4)	4(5.3)
Tinea cruris	17 (22.8)	0 (00)	2(2.6)	2(2.6)	6(8)	4(5.3)	3(4)
Tinea pedis	14 (18.6)	0 (00)	1(1.3)	3(4)	6(8)	2(2.6)	2(2.6)
Tinea capitis	3 (4)	0 (00)	0 (00)	1(1.3)	0(00)	2(2.6)	0(00)
Tinea barbae	3 (4)	0 (00)	1 (1.3)	0 (00)	0(00)	1(1.3)	1(1.3)
Tinea manuum	2 (2.6)	0 (00)	0 (00)	0 (00)	1(1.3)	0(00)	1(1.3)
Tinea unguium	2 (2.6)	0 (00)	0 (00)	1(1.3)	0(00)	0(00)	0(00)
Tinea faciei	1(1.3)	0 (00)	00	0(00)	00(00)	0(00)	0(00)
Pityriasis versicolor	3(4)	0 (00)	2 (2.6)	0(00)	1(1.3)	0(00)	0(00)
Piedra	1 (1.3)	0 (00)	0 (00)	1(1.3)	0(00)	0(00)	0(00)
Total n (%)	75 (100.0)	0 (00)	14(18.6)	16(21.3)	21(28)	13(17.3)	11(14.6)

Out of the total 75 samples 52 (69.4%) samples were positive for direct KOH examinatation while 23(30.6%) were negative. Out of the total 75 samples 59 (78.6%) were culture positive in which 48 (64%) were culture positive amongst KOH positive & 4(5.3%) were culture negative. Amongst the 23(30.6%) KOH negative samples 11(14.6%) were culture positive & 12 (16%) were culture negative. **(Table 3)**

Table 3: KOH and culture positivity of specimens

KOH results	No. of cases N (%)	Culture results	
		Positive N (%)	Negative N (%)
Positive	52(69.4)	48(64)	4(5.3)
Negative	23(30.6)	11(14.6)	12(16)
Total	75(100)	59(78.6)	16(21.3)

Out of 59 culture positive amongst the 75 samples, 41 (54.6%) dermatophytes and 28(37.3%) non dermatophytes were isolated amongst which *T. mentagrophytes* (27.1%) was the predominant isolate in dermatophytes followed by *T. rubrum* (16.9%), *T. tonsurans* and *M. audouinii* (8.5% each), *T. interdigitale* and *T. schoenleinii* (3.4% each) and *M. fulvum* from only one case. Among non-dermatophytes *Penicilium* (8.4%) was the most common isolate followed by Candida spp.and Malasezzia spp. (5.1% each), Aspergillus spp., *Fusariumoxyspora* and Alternaria spp. (3.4% each) and Exopjiala spinifera from one case only. The most common clinical manifestation in which maximum *T. mentagrophytes* isolated was of T. corporis 7 (11%), followed by Tinea pedis 5 (8.4%) and then Tinea cruris 3 (5%) and 1(1.6%) for T. capitis. The next isolate *T. rubrum* 10(16.5%) with maximum isolation from Tinea cruris 3(4%) followed by Tinea pedis 2 (2.6%) ,Tinea barbae 2 (2.6%) and 1(1.6%) from Tinea corporis , Tinea capitis and Tinea faciei each. **(Table 4)**

Table 4: Distribution of various fungal isolates from culture positive cases

linicaltypes	*Culture Positive*	Dermatophytes							Non dermatophytes						
		T. mentagrophytes	*T.rubrum*	*T.tonsurans*	*M. audouinii*	*T.interdigitale*	*T.schoenleinii*	*M. fulvum*	*Penicillium spp.*	*Aspergillus spp.*	*Candida spp.*	*Fusarium oxyspora*	*Alterneria spp.*	*Exophiala spinifera*	*Malassezia furfur*
Tinea corporis	21	7	1	2	2	0	1	1	1	2	0	2	2	0	0
Tineacruris	14	3	3	1	2	1	1	0	2	0	1	0	0	0	0
Tineapedis	11	5	2	1	1	1	0	0	1	0	0	0	0	0	0
Tineacapitis	3	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Tinea barbae	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0

Tinea manuum	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Tinea unguium	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Tineafaciei	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pityriasis versicolor	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Piedra	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Total (%)	59 (100)	16 (27.1)	10 (16.9)	5 (8.5)	5 (8.5)	2 (3.4)	2 (3.4)	1 (1.7)	5 (8.5)	2 (3.4)	3 (5)	2 (3.4)	2 (3.4)	1 (1.7)	3 (5)

Figure 1 a

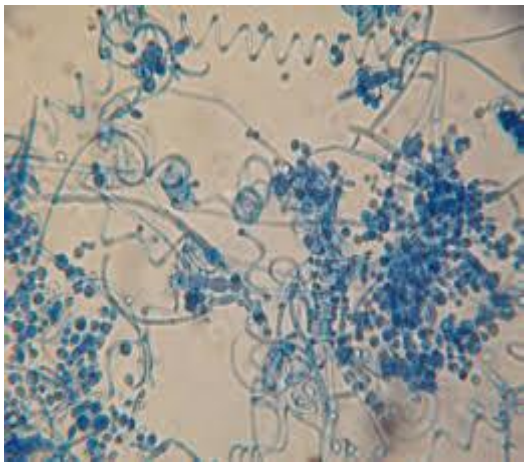

Figure 1 b

Fig.1 a. Flat, white, centrally folded, cottony colony of *T. mentagrophytes* on SDA; **b** Lactophenol Cotton Blue (LPCB) mount of *T. mentagrophytes* showing characteristics of spiral hyphae and clusters of microconidia.

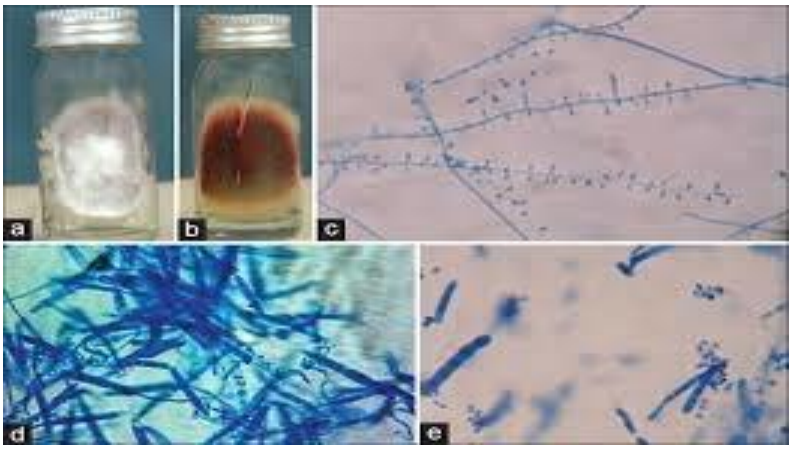

Fig. 2(a & b) Flat to raised, cream color, downy colony of *T .rubrum* on SDA with deep brown reverse pigmentation; **(c)**Tear drop microconidia arranged in 'birds on fence'pattern; **(d &e)** pencil shaped macroconidia in LPCB mount.

Figure 3 a

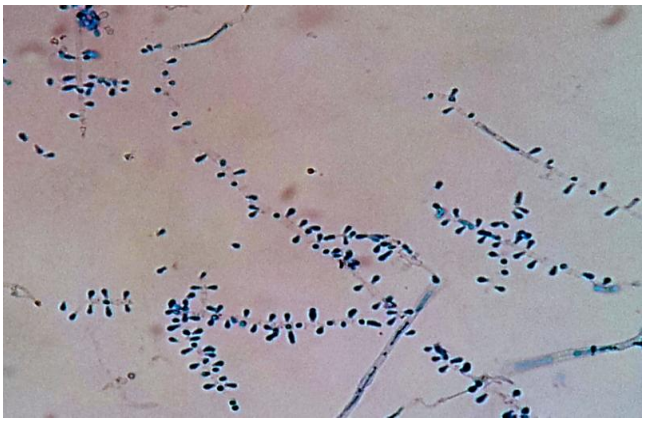

Figure 3 b

Figure 3 a. Powdery, yellowish brown, flat with raised center colony of *T. tonsurans* on SDA; **3 b.** LPCB mount of *T.tonsurans* showing characteristic microscopic findings- numerous tear shaped clavate microconidia borne singly or in clusters.

Figure 4 a

Figure 4b

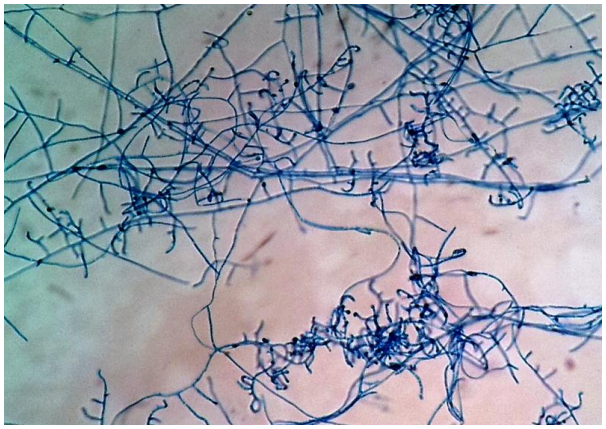

Figure 4 c

Figure 4(a-b): Irregular heaped, smooth white to cream colony with radiating grooves colony of *T. schoenleinii* on Sabouraud's dextrose agar with no reverse pigmentation**; (c)** LPCB mount of *T. schoenleinii* showing characteristic microscopic findings- antler "nail head" hyphae(black arrow, favic chandeliers) and lack of micro and macroconidia.

DISCUSSION

The present study was conducted to know the prevalence of fungal isolates in superficial mycoses cases in rural population attending tertiary care hospital in North India.

Out of total 75 cases of superficial mycoses, the most common clinical type encountered amongst the sample of 75 patients was of T. corporis 29(38.6%), which was predominant amongst the male patients 17(22.6%) where as 12 (16%) female patients were affected. T. corporis (38.6%) was the most common clinical type observed in cases followed by Tinea cruris (22.6%), T. pedis (18.6%). In concordance with the present study, T. corporis was also found to be the commonest presentation of superficial mycosis by Naglot A et al [6] from Tezpur, Assam, Sen and Rasul from Guwahati, Assam [7], Sharma R et al from Sikkim [8], Kumar S et al [9], Agarwal U S et al[10] and Mahajan S et al [11] from various parts of North India have also found Tinea Corporis to be the most common presentation of superficial mycosis in their respective studies. However, T. unguium has been found to be the second most commonest presentation in 20% studies in a systematic review done by Das S et al. [12]

Out of the 59 culture positive samples 46(72%) males and 13(28%) females were affected, with male female ratio of 2.5:1. Superficial mycoses which might be due to their higher physical activities associated with relatively higher sweaty outdoor activities and exposure to fungal infection than females (who mostly presented with cutaneous lesions in

intertriginous areas in this study). Similarly, male preponderance of superficial mycoses was also reported by studies carried out by majority (82.5%) of studies in a systematic review done by Das S et al. [12] The lower prevalence in females might be due to non-reporting to health care facilities from semi-urban and rural areas due to ignorance and social stigmas or due to over-the-counter use of topical steroids.

The most common age group encountered was among 31-40 years 21(28%). Our findings are a bit different from majority of studies who report highest prevalence among 21-30 years age group. [12] However Rajamohanan R *et al.*[13] from Puducherry and Sharma Y et al [14] from Delhi have reported that the commonest age group in their study was 31-40 years with prevalence rate of 29.6% and 31.25% respectively.

Out of the total 75 patients enrolled, 52(69.33%) were positive and 23 (30.6%) in direct microscopic examination (KOH) while 59 (78.6%) were culture positive and 16(21.3%) were culture negative. Bindu and Pavithran [15] reported similar KOH positivity of 64% while Mistry MA *et al.*[16], (86.86%) and Ghosh RR *et al.*[17], (91.34%) reported with higher KOH positivity as compared to our study. Santosh HK *et al.* [18] have reported with low KOH positivity of 55.37% as compared to our study. This may be due to non-viability of the fungi due to application of antifungal agent prior to sample collection or could be due to absence of fungal element in the portion of sample used for culture. Ghosh RR *et al.*[17], (87.43%) and Jagdeesan M *et al* [19] (74.3%) have reported similar high culture positivity compared to our study while Bindu and Pavithran [15] (45.3%) ; Santosh HK *et al.*[18], (46.97%); Nilekar SL *et al.*[20], (45.62%) and Tokbigi Phudang R *et al* [21](45%) have reported lower culture positivity rate.

Among 23 (30.6%) KOH negative samples 11(14.6%) were culture positive, while 12 (16%) were negative which shows importance of culture and direct microscopy in diagnosing cases of superficial mycoses.

In present study, *T. mentagrophytes* (21.3%) was the predominant isolate followed by T. *rubrum* (13.3%), *T. tonsurans* (6.6%), *T. schoenleinii* (2.6%), M. *audouinii* (6.6%) and *M. fulvum* (1.3%). This finding has been seconded by a systematic review by Das S *et al.*[12] who have reported a consistent rising trend of *T. mentagrophytes* since 2015 all over India. However, one of the author of current study - Sahai and Mishra[22] reported first emergence of *T. mentagrophytes* in central India in 2011. *Trichophyton rubrum* was found to be the dominant causative organism in superficial mycoses as well as dermatophytosis before 2015 by reports from Naglot A et al [6] from Tezpur, Assam, Sen and Rasul from Guwahati, Assam [7], Sharma R et al from Sikkim [8], Kumar S et al [9], Agarwal US et al[10] and Mahajan S et al [11] from various parts of North India, Lakshmanan A et al [22] from Chennai, Bindu and Pavithran [23] from Calicut, Lavanya and Solabannavar [24] and Surendran K et al [25] from Karnataka. In the contrast, *T. tonsurans* was the commonest isolate from superficial mycoses in report of Wg Cdr Sanjiv Grover and Lt Col P Roy [26] from Northeast India.

We did not isolate any *Epidermophyton spp*. in this study which might be due to low sample size. Uncommon species of *M. fulvum* and *T. interdigitale* has been found. These species have been reported for the first time in our region to the best of our knowledge. These are geophilic fungus associated to cause rare human infections.

Low sample size and hospital based study have been limitations of our study and a larger cross sectional study of rural population is needed to confirm our findings.

CONCLUSION

Thus, to conclude, superficial mycoses are an extremely common health condition in India. The epidemiological and mycological pattern of superficial mycoses showed a fairly consistent trend across various regions of India. Dermatophytes were the main causative agents of superficial mycoses; the most common species were *T. mentagrophytes* and *T. rubrum.*

Isolation of rare species like M. *fulvum, T. interdigitale* and *Exophiala spinifera* confirms our findings of change in distribution of dermatophytes in our region. India is a growing economy and during last few decades interstate migration of population and increase in national and foreign tourism might be an important reason for change in frequency of dermatophytes and uncommon fungal isolates in clinical practice. To diagnose such cases medical mycologist need to be updated with the current scientific literature.

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