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Original Article

Clinical study on antifungal drug resistance among cases of dermatophytosis in patients reporting to multiple tertiary care hospitals

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ABSTRACT

Background: In a tropical country like India, the warm and humid climate plays an important role in the increased incidence of superficial fungal infections. This is a study to identify the causative fungi of dermatophytosis and their in vitro antifungal susceptibility pattern among patients reporting to multiple tertiary care hospitals.

Methods: Skin scrapping, nail clipping, and hair follicles were processed for microscopy, culture, and antifungal susceptibility testing as per standard guidelines. Antifungal susceptibility was performed as per published by Clinical Laboratory Standards Institute for yeasts (M27−A3) and filamentous fungi (M38−A2).

Result: The study sample had a predominantly male population with the commonest age group being 21−30 years (39.57%) followed by 31−40 years (31.46%). Tinea corporis (57.30%) was the most common clinical presentation followed by tinea cruris (20.85%) and onychomycosis (14.73%). Microscopy positivity was 43.19%, while culture positivity was 23.97%. Dermatophytes accounted for the majority of isolates. All fungal isolates had high minimum inhibitory concentration (MIC) to fluconazole, suggesting that dermatophytes are possibly resistant to this drug.

Conclusion: Trichophyton mentagrophytes is confirmed as the dominant pathogen of dermatophytosis in all three tertiary care hospitals.

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Introduction

Among fungal infections, dermatophytosis has a high prevalence, especially due to the ease of travelling[1] and various medical comorbidities.[2] Dermatophytosis is superficial skin infection with aetiological agents either being dermatophytes or non-dermatophytes. As per WHO, the worldwide prevalence of superficial fungal infections was found to be 20–25%.[3] The distribution of dermatomycoses, their aetiological agent, and infection pattern depends on the geographic distribution, environmental conditions, and cultural factors.[4] In a tropical country like India, the warm and humid climate plays a role in higher incidence of superficial fungal infections.[4] Furthermore, other factors such as poor socioeconomic conditions, pets, overcrowding, and sharing of towels are also associated with a higher dermatophytes infection rate.[5]

Due to the widespread use of over-the-counter local steroid and antifungal ointment, clinical presentation is often atypical and requires laboratory confirmation.[6] The antifungal treatment guidelines are based on the aetiological agent, site, and extent of the lesion and antifungal susceptibility pattern.[7] Both topical and systemic antifungal agents can be used for the treatment of dermatophytosis. The commonly used ones are allylamines (terbinafine), triazoles (fluconazole, itraconazole, and voriconazole), imidazoles (ketoconazole), and griseofulvin.[7]

Although dermatomycoses respond to conventional drugs, they tend to recur.[6–8] Antifungal susceptibility testing is essential for detecting drug resistance among various fungal species and selecting an effective treatment.[7,8] Antifungal susceptibility testing is performed by determining the minimum inhibitory concentration (MIC) of the antifungal agents based on the micro broth dilution method described by the Clinical Laboratory Standards Institute (CLSI) for yeasts (M27 – A3)[9] and filamentous fungi (M38 – A2).[10]

This multicentric study was conducted to identify the fungal species causing dermatophytosis, along with its antifungal susceptibility testing pattern, of isolates from Armed Forces personnel.

Material and methods

This study was multicentric of two years duration. The study population was patients presenting with clinical features of dermatophytosis at multiple tertiary care centres. Following the exclusion of the immunosuppressed patients, a total population sample size of 801 (267 for each centre of the three tertiary care centres) was included in this study. Patient samples were skin scrapings, nail clippings, and plucked hairs, which were collected on the black paper and transported to the laboratory in sterilized universal containers.

Direct microscopy with hydrogen peroxide (KOH) was utilized for microscopic detection of fungal elements. KOH at varying concentrations (10%–30%) was used as a keratin digesting agent, hence facilitating easy detection of the fungal elements. Growth from slide cultures was identified up to the species level utilizing direct microscopy and biochemical reactions.[8]

Antifungal susceptibility testing for dermatophytes and other fungus species was conducted using micro broth dilution methods as described in CLSI documents.[9,10] Antifungal drugs and their dilutions used are shown in Table 1. Controls used were *Candida parapsilosis* (ATCC®22,019), *Candida krusei* (ATCC®6258), *Fusarium solani* (ATCC® 3636), and *Trichophyton mentagrophytes* (ATCC®MYA4439).

Minimum inhibition concentration, MIC 50 and MIC 90, and geographical mean of various antifungals were determined. A written informed consent was taken from all patients. The study was approved by the Institutional Ethics Committee.

Results

Patient's age range was from 18 years to 56 years (mean ± SD = 32.51 ± 9.88). The commonest age categories were 21–30 years (39.57%), followed by 31–40 years, 41–50 years and 11–20 years, respectively. Gender distribution was male 99.75% and female 0.25%. Over-the-counter, antifungal ointment exposure was documented in 67.79% of the study population.

Clinical patterns

The most common clinical presentation was tinea corporis (57.30%) followed by tinea cruris (20.85%). Cases of onychomycosis, tinea pedis, tinea manuum, and tinea barbae were also identified (Table 2). Nondermatophyte skin lesions included tinea corporis, tinea cruris, and onychomycosis (Table 3).

Microscopy and culture

Out of the total of 801 samples processed, KOH mount was positive in 346 samples (43.19%), whereas slide culture was positive in 192 cases (23.97%). Most common dermatophytes isolates was *T. mentagrophytes*, followed by *Microsporum canis*, *Microsporum gypseum*, *Microsporum audouinii Trichophyton rubrum,* and *F. solani* in this order (Table 4). Nondermatophytic fungi isolated were *Candida tropicalis*, *C. parapsilosis*, and *Sporothrix schenckii* (Fig. 1).

Antifungal susceptibility test by broth microdilution

In vitro antifungal susceptibility testing (AFST) was performed on 192 isolates of dermatophytes, 10 isolates of Candida species and 2 isolates of *F. solani*. *S. schenckii* was lost in

Table 1 – Antifungal dilutions used for dermatophytes and non dermatophytes (CLSI 2017).

Antifungal	Dermatophytes Dilutions used	Nondermatophytic fungi Dilutions used
Posaconazole	0.004–8 μg/ml	0.0313–16 μg/ml
Itraconazole	0.001–0.5 μg/ml	0.0313–16 μg/ml
Voriconazole	0.001–0.5 μg/ml	0.0313–16 μg/ml
Griseofulvin	0.125–64 μg/ml	–
Terbinafine	0.001–0.5 μg/ml	–
Amphotericin B	–	0.0313–16 μg/ml
Anidulafungin	–	0.015–8 μg/ml
Caspofungin	–	0.015–8 μg/ml

Table 2 – Clinical type, KOH mount, and fungal culture correlation.

Clinical type	Total no of cases	No. of KOH positive	No. of culture positive
Tinea corporis	459	210 (45.75%)	110 (23.96%)
Tinea cruris	167	86 (51.49%)	40 (23.95%)
Onychomycosis	119	22 (18.48%)	23 (19.32%)
Tinea pedis	24	12 (50%)	9 (37.50%)
Tinea faciae	12	2 (16.67%)	4 (33.34%)
Tinea barbae	4	2 (50%)	2 (50%)
Tinea incognito	5	5 (100%)	0
Tinea mannus	5	3 (60%)	3 (60%)
Tinea capitis	3	3 (100%)	3 (100%)
Paronychia	3	0	0
Sporotrichosis	1	1 (100%)	1 (100%)
Total	801	346	192

subculture. Antifungal agents tested for each fungus species demonstrated a wide MIC range and geographical mean (Table 4), whereas MIC 50 and MIC 90 also showed marked variation (Tables 5–7).

Discussion

In our study, the mean age was 32.51 years (standard deviation ± 9.88 years), and age group distribution was similar to those reported by the previous Indian authors.[11–13]

In this study, the male preponderance was due to increased physical activity and sweating among male population. Similarly, many studies have also reported dermatomycosis incidence being higher in males due to greater physical activity and increased sweating.[14]

Among the clinical manifestations, tinea corporis was the most common, followed by tinea cruris and onychomycosis. These findings are similar to those reported by Bindu et al study.[15] Regular and prolonged use of shoes and socks by soldiers, further contributed to dampness and warmth and thus could be the reason why tinea pedis is very common in them. This rationale has been documented by a few authors.[14,15] In this study, a single case of sporotrichosis was confirmed by histopathological examination as well as culture. In India, this infection is more prevalent in the sub- Himalayan belt where the prevalence ranged from 23% to 40%.[15]

Direct microscopy findings were similar to those of earlier published literature.[16] Nonvisualization of hyphae on direct microscopy was due to masking by the inflammatory cells.[16]

In this study, culture positivity was 23.97%, however, in literature it has varied from 8.6% to 97%. This wide range can be due to variation in the etiological agents, detection method, and previous exposure to antifungal formulations.[13–16] Among etiological agents, dermatophytes accounted for the majority of cases, followed by Candida and other nondermatophytes, this result is similar to other studies.[14–16] Among the dermatophytes, the most common isolated genera were *Trichophyton* spp. (77.16%) followed by *Microsporum spp*. (22.8%).

At the species level, the most predominant dermatophytes were *T. mentagrophytes* (Fig. 2) followed by *M. canis* and *M. gypseum*. This finding though at variance with some studies, which reported *T. rubrum* as most common,[3,5,12,13,16]; however, it was in accordance with other published studies.[17–19] This can be explained by the fact that *T. rubrum* is generally linked to chronic dermatophytosis and robust medical facility in the army medical corps ensures timely identification and management.[17–19]

Table 3 – Association between clinical type and etiological agent.

Clinical type	Total culture positives	Etiological agent
Tinea corporis	110	*T. mentagrophytes* (83) *M. gypseum* (17) *M. canis* (8) *C. parapsilosis* (5) *C. tropicalis* (5) *A. glaucus* (3) *Cladosporium* (6) *Nigrospora spherical* (6)
Tinea cruris	40	*T. mentagrophytes* (37) *M. canis* (9) *M. gypseum* (3) *M. audouinii* (3) *C. parapsilosis* (3) *C. tropicalis* (5) *A. glaucus* (3)
Onychomycosis	23	*T. mentagrophytes* (9) *M. gypseum* (3) *C. tropicalis* (5) *C. parapsilosis* (3) *A. glaucus* (3)
Tinea pedis	9	*T. mentagrophytes* (5) *T. rubrum* (2) *F. solani* (2)
Tinea faciei	4	*T. mentagrophytes* (4)
Tinea barbae	2	*T. mentagrophytes* (2)
Tinea manuum	3	*T. mentagrophytes* (3)
Tinea capitis	3	*M. canis* (3)
Sporotrichosis	1	*Sporothrix schenckii* (1)

Table 4 – Summary of AFST patterns of different isolates (CLSI 2017).

Fungi	Number of Isolates	Antifungal agent	MIC range (μg/ml)	Geometric mean
T. mentagrophytes	125	Terbinafine	0.0019–4	0.010
		Griseofulvin	0.125–0.5	0.205
		Itraconazole	0.0009–2	0.013
		Posaconazole	0.0019–1	0.021
		Voriconazole	0.0019–1	0.034
M. canis	20	Terbinafine	0.0019–0.0625	0.010
		Griseofulvin	0.125–0.5	0.410
		Itraconazole	0.0078–0.5	0.051
		Posaconazole	0.0019–0.25	0.011
		Voriconazole	0.0039–0.0625	0.025
M. gypseum	17	Terbinafine	0.0019–0.125	0.012
		Griseofulvin	0.125–1	0.397
		Itraconazole	0.0078–2	0.25
		Posaconazole	0.0019–1	0.099
		Voriconazole	0.0313–0.25	0.0.070
C. parapsilosis	5	Amphotericin B	0.25	
		Itraconazole	0.5	
		Voriconazole	0.125 (4); 0.5 (1)	
		Posaconazole	0.0625 (4); 0.25 (1)	
		Anidulafungin	0.5 (5)	
		Caspofungin	0.25 (4); 0.5 (1)	
C. tropicalis	5	Amphotericin B	0.25	
		Itraconazole	0.125 (4); 0.5 (1)	
		Voriconazole	0.0313(1); 0.125 (4)	
		Posaconazole	0.0313(1); 0.5(4)	
		Anidulafungin	0.0625; 0.125	
		Caspofungin	0.125; 0.25	
F. solani	2	Terbinafine	>16	
		Itraconazole	>16	
		Posaconazole	16	
		Voriconazole	>16	
		Anidulafungin	16	
		Caspofungin	>16	
		Amphotericin B	2	

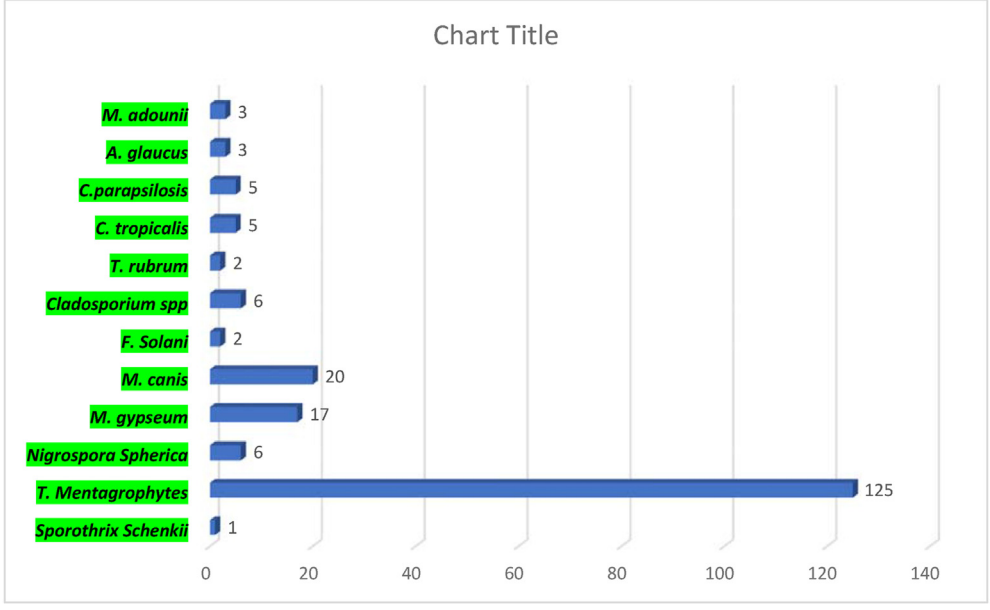

Fig. 1 – Bar chart showing number of fungal species isolated on culture (total-192).

Table 5 – MIC 50, MIC 90, and geometric mean of terbinafine and itraconazole.

Species	Terbinafine/Itraconazole MIC 50 (μg/ml)	Terbinafine/Itraconazole MIC 90 (μg/ml)	Geometric mean
T. mentagrophytes (n = 125)	0.008/0.016	0.125/0.0625	0.010/0.013
M. canis (n = 20)	0.016/0.0313	0.016/0.25	0.010/0.05
M. gypseum (n = 17)	0.008/0.25	0.016/1	0.010/0.025

Table 6 – MIC 50, MIC 90 and geometric mean of Voriconazole and Posaconazole.

Species	Voriconazole/Posaconazole MIC 50 (μg/ml)	Voriconazole/Posaconazole MIC 90 (μg/ml)	Geometric mean
T. mentagrophytes (n = 125)	0.0313/0.016	0.5/0.125	0.034/0.021
M. canis (n = 20)	0.0313/0.008	0.25/0.0625	0.025/0.011
M. gypseum (n = 17)	0.0313/0.0625	1/0.25	0.070/0.099

Table 7 – MIC 50, MIC 90, and geometric mean of fluconazole.

Species	Fluconazole MIC 50 (μg/ml)	Fluconazole MIC 90 (μg/ml)	Geometric mean
T. mentagrophytes (n = 49)	32	64	36
M. canis (n = 7)	32	64	35
M. gypseum (n = 6)	32	64	36

The higher isolation rate of *T. mentagrophytes* may be due to the changing trend in the etiological agents of dermatophytes in the country.[19] Similarly, in this study, *M. canis* and *M. gypseum* were commonly identified as etiological agents; however, no *Trichophyton tonsurans*, *Trichophyton violaceum*, *Trichophyton verrucosum*, or *Epidermophyton floccosum* was isolated from any skin lesions. These observations are again in alignment with the changing epidemiology, with progressive decline in the etiological role of *T. verrucosum* and *E. floccosum*.[19] Nondermatophytic fungi isolate recovered as etiological agents are similar to those reported by other studies, and it includes *Candia spp*, *F. solani*, and *S. schenckii*.[20]

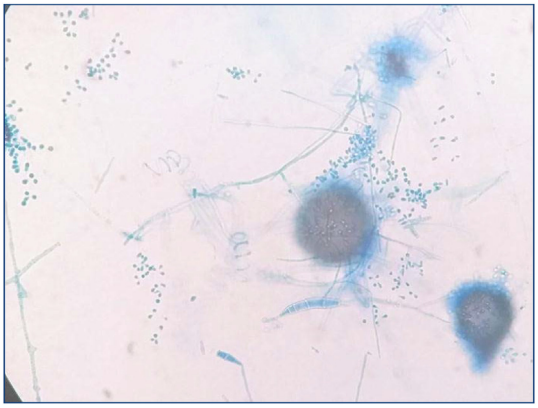

Fig. 2 – Slide culture of *T. mentagrophytes* showing pencil-shaped macroconidia, grape-like clusters of microconidia and spiral hyphae.

Analysis of MIC mean, MIC 50 and MIC 90 of terbinafine, itraconazole, fluconazole, voriconazole, and posaconazole showed reduced efficiency of terbinafine and voriconazole against *M. canis* and reduced efficiency of itraconazole for *M. gypseum*. Whereas, all *T. mentagrophytes* isolates demonstrated uniform sensitivity across all tested antifungals. Among azoles, fluconazole showed an emerging resistance as its MIC 50 was closer to the mean MIC for all fungal species; however, Voriconazole and Posaconazole mostly had very low MIC levels (Tables 5–7). These findings are in concurrence with reports from other researchers.[9–20]

This study's antifungal susceptibility testing results correlated well with the previously published national and international studies.[9–20] Our study has shown that all *T. mentagrophytes* isolates had low MIC to terbinafine and Itraconazole and high MIC to fluconazole, whereas *M. canis and M. gypseum* isolates had low MIC for Griseofulvin. It is therefore recommended to use tropical terbinafine application for *Trichophyton spp*. and Griseofulvin for *Microsporium spp* skin lesions. These recommendations are identical to the existing international guidelines.[20]

Disclosure of competing interest

The authors have none to declare.

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