Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i6.6630

SPECIES IDENTIFICATION AND DIFFERENTIATION OF DERMATOPHYTES, ANTIFUNGAL SUSCEPTIBILITY PROFILE FROM A TERTIARY CARE CENTRE IN CENTRAL INDIA

Sakshi Vishnoi[1*], Madhurendra Singh Rajput[2]

[1]*PhD Scholar, Dept. of Microbiology, Malwanchal University, Indore, Madhya Pradesh, India
[2]Professor, Dept. of Microbiology, Malwanchal University, Indore, Madhya Pradesh, India

***Corresponding author:** Sakshi Vishnoi
*Email: Sakshivishnoi0003@gmail.com

Abstract

Objective: One of the oldest disease, dermatophytosis, is growing more common in the modern era. Because of this, understanding antifungal susceptibility has become crucial in the modern era..

Material and Methods: This 18-month prospective study included all dermatophytes that were isolated during that time. Dermatophytes were discovered using standard phenotypic techniques. As per the Clinical Laboratory Standard Institute M38A2, antifungal susceptibility testing was carried out for griseofulvin, terbinafine, and itraconazole, and minimum inhibitory concentrations (MICs) were determined within 5 days.

Results: Information on the patient and related risk factors were noted. Of the 320 patients with dermatophytosis of the skin, 79.3% had fixed dose combination medications with steroids. 303 of the 272 dermatophytes that were isolated throughout the study period came from skin scrapings, and 48 came from nail samples. Presentations of tinea corporis with cruris were the most frequent. *Trichophyton mentagrophytes complex* (40.3%)was the most often isolated dermatophyte from skin scrapings (129 out of 272), whereas *Trichophyton rubrum complex* (66%) ,(32 out of 48) was recovered from nail samples. Itraconazole had the lowest MICs, followed by terbinafine and griseofulvin, according to the MIC50 and MIC90 data. In summary Antifungal resistance and species distribution epidemiology are evolving, necessitating ongoing monitoring of these dermatophyte characteristics.

Keywords: ►Dermatophytes ►Trichophyton ►antifungal susceptibility ►Griseofulvin ►Terbinafine

Introduction

Millions of superficial fungal infections are annually observed in humans. The majority of this mycosis is caused by dermatophytes which develop in the dead part of keratinized tissue of the stratum corneum, within and around hairs, nails and on the skin [(1)]. According to World Health Organization the worldwide prevalence of dermatophytes is 25% and estimated that from 30 to 70% of adults are asymptomatic hosts of these pathogens and that the incidence of the disease increases with age. [(2)] It is very important for physician to understand the different clinical findings and the associated pathogen for effective treatment, [(3)] Despite the availability of a wide range of antifungal agents for therapeutic purposes, the failure in treatment has been extensively reported. This may be multifactorial and the reasons include the severity of the dermatophytosis, causative agents, patient

co-morbidities such as immune suppressed patients and some antifungal drugs may modify blood levels, inappropriate or insufficient drug administration, discontinuation of therapy and noncompliance of the patient. [4,5,6]

Material and Methods

This prospective observational study was carried out at the Centre Mycology section of microbiology laboratory of Index Medical College Hospital and Research Centre, Indore (M.P.) India, in cooperation with the departments of dermatology, venereology, and leprosy. The study was carried out over the course of 18 months, from January 2021 to August 2022. The study comprised a total of 320 samples (272 skin scraping samples and 48 nail samples) that tested positive for dermatophytes in fungal cultures and potassium hydroxide (KOH) mounts. No hair sample was obtained in this time frame. Following the patient's informed consent, a thorough case history, examination, and other pertinent workup were completed and recorded on a proforma. Clinical specimens were treated with KOH on a slide or tube and looked for thin, septate-branched hyphae or chains of arthroconidia. On Sabouraud dextrose agar (SDA, HiMedia, India) tube slants, samples were inoculated with gentamicin, chloramphenicol, and cycloheximide. Inoculating duplicates of each medium, they were then incubated at 25°C and 37°C. For the next three weeks, fungal growth was monitored twice a week and every day for the first week. The Mold-growing tube was recognized using normal mycological techniques, microscopic examination using lactophenol cotton blue mount and slide cultures, and morphological parameters such as growth rate, texture, and colour of the colony on the obverse and reverse of SDA.[7]For griseofulvin, terbinafine, and itraconazole, antifungal susceptibility testing (AFST) was carried out using the microbroth dilution technique in accordance with the Clinical Laboratory Standard Institute's (CLSI) "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi" (M38 A2). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were utilized as quality strain. The antifungals under test were obtained commercially from Sigma-Aldrich in China as powders. The reading of minimum inhibitory concentrations (MICs) following five days at 35°C of incubation. The minimum inhibitory concentration (MIC) for the medications griseofulvin, terbinafine, and itraconazole was determined by comparing the concentration of the antimicrobial agent that produced an 80% or higher growth reduction to growth control (drug-free media containing inoculum). [8] The institute's ethics committee gave its approval to the study that was carried out.

Result -Tinea corporis (plates 8a–8f) was the most prevalent type of infection among the 320 cases of dermatophyte infection in the current study, accounting for 159 (49.68%) cases, of which 67 (42%) were male and 92 (57%) were female. This was followed by *Tinea cruris* (plates 9a–9f), accounting for 97 (30.31%) cases, of which 69 (71%) were male and 28 (28.8%) were female. 48 (15%) cases of *Tinea unguium* (plates 10a–10f) were observed; of them, 18 (37.5%) were male and 30 (62.5%) were female. Out of the 11 (3.4%) *Tinea faciei* patients (plates 11a–11f) 2(18%) were female and 9(81.8%) were male . Of the 5 (1.5%) cases of *Tinea pedis* (plates 12a–12e), 3 (60%) were female and 2 (40%) were male. There were 41 occurrences of Tinea corporis, most of which were in the age range of 21 to 30. The age group of 31-40 years old had the highest number of *Tinea cruris* cases, totalling 26. With 61 instances, *Tinea unguium* was most common in the age range of 31 to 40. (**Table No -1**)

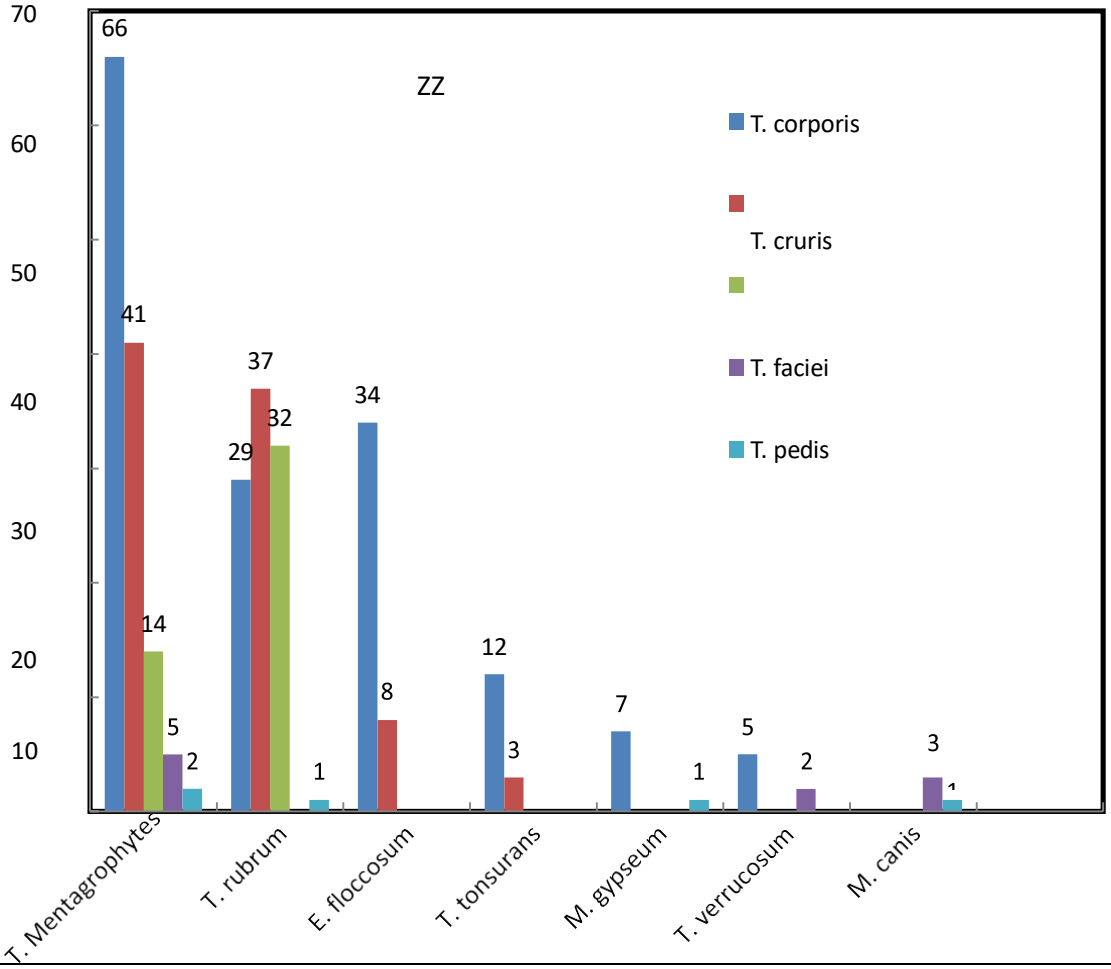
In the present study total 320 cases of dermatophyte infection were included. A total of 320 instances of dermatophyte infection were included in the current investigation. With 129 (40.3%) isolations, *Trichophyton mentagrophytes* was the most frequently isolated species. Of these, 66 instances were of the Tinea corporis type, 41 cases were of the Tinea cruris type, 14 cases were of the *Tinea unguium* type, 5 cases were of the *Tinea faciei* type, and 2 cases were of the *Tinea pedis* type. With 98 (30.6%) cases, Tinea *rubrum* type was the second most often isolated species. There were only 1 *Tinea pedis* type, 29 *Tinea corporis* type, 37 *Tinea cruris* type, and 32 *Tinea unguium* type isolations. 42 (13.1%) subjects had *Epidermophyton floccosum* isolated from them, 34 of which had *Tinea corporis* type and 8 of which had *Tinea cruris* type. 12 of the 15 participants (4.6%) who had *Tinea corporis* and 3 of the subjects who had Tinea cruris had *Trichophyton tonsurans* isolated from them. 8 individuals

(2.5%) had *Microsporum gypseum* isolated from them; 7 of the subjects had *Tinea corporis* type, whereas only 1 had *Tinea pedis* type. 7 participants (2.1%) were found to have *Trichophyton verrucosum*; 2 of these cases were *Tinea faciei* and 5 of the *Tinea corporis* types. 4 patients (1.2%) were found to have *Microsporum canis*; 3 belonged to the *Tinea faciei* type and 1 to the *Tinea pedis* type.. **(shown in chart fig.1)**

Table 1. Age and gender wise distribution of subjects according to clinical type infection

Clinical types and gender										
	***T. corporis* (on glabrous skin)**		***T. cruris* (on groin area)**		***T. unguium* (on nails)**		***T. faciei* (on face)**		***T. pedis* (on feet)**	
Age group (year)	**F**	**M**	**F**	**M**	**M**	**F**	**M**	**F**	**M**	**F**
0-10	-	1	-	-	-	-	2	-	-	-
11-20	12	3	14	1	-	-	1	-	1	-
21-30	19	22	15	6	7	5	3	1	-	2
31-40	11	24	17	9	2	14	3	-	-	1
41-50	10	22	10	8	4	7	-	-	1	-
51-60	9	12	10	3	3	3	-	1	-	-
61-70	6	8	3	1	2	1	-	-	-	-
Total	67	92	69	28	18	30	9	2	2	3
Percentage	159 (49.68%)		97 (30.31%)		48 (15%)		11 (3.4%)		5 (1.5%)	
χ^2 *p. value*	1.63 0.95		1.22 0.99		1.1 0.99		0.27 0.97		0.47 0.97	

Figure 1. Isolated dermatophyte species in relation to their clinical types



AFST was done by themicro-broth dilution method according to the CLSI document M38-A2. The AFST of *T. mentagrophytes* complex for griseofulvin, terbinafine, and itraconazole showed the MIC range of 0.25 to 64, 0.0156 to 4, and 0.0312 to0.125 µg/mL, respectively. AFST of *T. rubrum* complex for griseofulvin, terbinafine, and itraconazole showed the MIC range of 0.25 to 128, 0.0156 to 2, and 0.03125 to 16 µg/mL, respectively **(Table 2)**

Table 2 MIC distribution of dermatophytes isolated from skin and nail samples

Isolates	MIC (µg/mL)	Griseofulvin			Terbinafine			Itraconazole		
Trichophyton mentagrophytes complex		Total	Dermatophytosis of skin	Tinea unguium	Total	Dermatophytosis of skin	Tinea unguium	Total	Dermatophytosis of skin	Tinea unguium
	Numbers	129	115	14	129	115	14	129	115	14
	MIC range	0.25—64	0.25–64	0.5–1	0.0156—4	0.0156—4	0.125—0.5	0.0312—0.125	0.0312—0.125	0.03125–0.0625
	MIC_{50}	1	1	0.5	0.25	0.25	0.25	0.0312	0.0312	0.0625
	MIC_{90}	4	4	0.5	4	4	0.5	0.0625	0.0625	0.0625
	GM	0.868	0.888	—	0.210	0.207	—	0.038	0.037	—
Trichophyton rubrum complex	Numbers	98	66	32	98	66	32	98	66	32
	MIC range	0.25—128	0.25–128	0.25—0.5	0.0156—2	0.0156—2	0.0156—0.125	0.03125—16	0.03125–16	0.0312
	MIC_{50}	0.25	0.25	0.25	0.0156	0.03125	0.0156	0.03125	0.03125	0.03125
	MIC_{90}	1	128	0.5	0.25	2	0.0156	0.03125	0.03125	0.03125
	GM	0.564	1.059	0.283	0.036	0.066	0.019	0.041	0.052	0.031
Trichophyton species	Numbers	22	22	0	22	22	0	22	22	0
	MIC range	0.5	0.5	—	0.0312–2	0.0312—2	—	0.03125–0.0625	0.03125–0.0625	—
	MIC_{50}	0.5	0.05	—	0.0625	0.0625	—	0.03125	0.03125	—
	MIC_{90}	0.5	0.5	—	2	2	—	0.0625	0.0625	—
	GM	0.5	0.5	—	0.177	0.177	—	0.037	0.037	—
Microsporum gypseum	Number	8	8	0	8	8	0	8	8	0
	MIC	0.25	0.25	—	0.0156	0.0156	—	0.0625	0.0625	—

Discussion - According to Al-Janabi (2014), dermatophytes are a special class of fungus that can infiltrate keratinized human and animal tissue, resulting in infections of the skin, hair, and nails that are frequently referred to as "ringworm" and "tinea." [(9)] According to Kumar (2017) and Khade (2018), Tinea infections were most common in the age range of 21–30 years old, then in the age group of 31–40 years old. [(10,11)] In line with the current investigation, Verma and Madhu (2017) also found that females between the ages of 31 and 40 are more susceptible to *Tinea corporis* and *Cruris.* Women were found to have dermatophyte infections under their folded breasts more frequently than men in this study. Young guys' increased dermatophyte infection prevalence can be attributed to changes in their hormone rhythms, increased sweating, and excessive physical activity.[(12)] According to Konda et al. (2017) and Doddamani et al. (2013), men in the 21–30 age range were also shown to be more physically active than females, which increased their risk of dermatophyte infection.[(13,14)] According to Kannan et al. (2006) and Konda et al. (2017), *Tinea corporis* was the most frequent fungal infection globally, with a prevalence of 20–25% for dermatophyte infections. [(15,13)] Kannan P., Janaki C. and Selvi G. S. (2006). Prevalence of Similarly, *Tinea corporis* was more common in males whereas *Tinea unguium* was more common in females in the current investigation. In line with this work, a number of other authors have also reported that human dermatophyte infections are primarily caused by anthropophilic fungus. [(16,)] Singal and Khanna (2011) noted that factors contributing to a higher incidence of onychomycosis include ageing populations, climates, occupations, immunosuppressive medication, sporting activities, walking barefoot, working with chemicals, and occlusive, ill-fitting footwear. [(17)]In this investigation, AFST was carried out using the microbroth dilution method for three drugs: griseofulvin, terbinafine, and itraconazole. After five days of incubation at 35°C, readings were collected. The time and temperature at which certain investigations are incubated vary greatly.

The majority of research have been conducted at 35°C, yet the duration of the incubation is inconsistent CLSI M38-A2 recommends incubation at.[18,19,20,21,22,]Over a period of four days, at 35°C, the growth control well exhibits sufficient (consistent) growth.[8] Itraconazole had the lowest MICs (MIC90 of 0.0625 µg/mL and 0.03125 µg/mL, respectively) for the *T. mentagrophytes complex* and the *T. rubrum complex* in this investigation. For the *T. mentagrophytes complex,* griseofulvin and terbinafine both provided a MIC90 of 4 µg/mL, whereas the MIC50 for the *T. rubrum complex* was greater than griseofulvin.

In summary, itraconazole has the lowest MICs, followed by terbinafine and griseofulvin, according to the MIC50 and MIC90 data. Most authors have observed similar trends.[23,24,25,26,27] However, Singh et al. have reported a larger MIC90 for terbinafine than griseofulvin, while Salehi et al. have demonstrated a higher MIC90 of as opposed to terbinafine.[27] In this investigation, Griseofulvin demonstrated a high MIC90 of 4 µg/mL for the *T. mentagrophytes complex* and 1 µg/mL for the *T. rubrum complex*. While very high MIC90 of 64 and 128 µg/mL have been reported by Rudramurthy et al. and Pathania et al., respectively, relatively few investigations show similar values.[24,19,23,25] While terbinafine remains the most promising treatment for dermatophytosis, it has become less effective over time.[28,29] For the *T. mentagrophytes complex,* we measured MIC90 values of 4 µg/mL, and for the *T. rubrum complex,* 0.25 µg/mL. While some studies have values similar to our study, the majority of studies [27,26,21,22] have reported values lower than this study.[23,25] A fairly high MIC90 of 32 has been observed by Singh et al. For terbinafine, µg/mL.28 In this investigation, itraconazole had the lowest minimum inhibitory concentrations (MICs) for *T. mentagrophytes complex* at 0.0625 µg/mL and *T. rubrum complex* at 0.0312 µg/mL. These values agree with the majority of previous research. [23,24,25,26] Badali et al. have found very high values of MIC90 of 16 µg/mL for itraconazole.[22]

Reference

1. Richardson M. D. and Warnock D. W. (2012). Fungal infection: diagnosis and management. John Wiley and Sons.
2. Sharma V., Kumawat T. K., Sharma A., Seth R. and Chandra S. (2015). Dermatophytes: Diagnosis of dermatophytosis and its treatment. African Journal of Microbiology, 9 (19): 1286-93.
3. Poyyamozhi J. S. and Lakshmanan A. (2018). Profile of dermatophyte infectionsamong rural population: A facility based prospective observational study. *International Journal of Community Medicine and Public Health*, 5: 1354-1359.
4. Bhatia V. K. and Sharma P. C. (2015). Determination of minimum inhibitory concentrations of itraconazole, terbinafine and ketoconazole againstdermatophyte species by broth microdilution method. *Indian journal of medical microbiology*, 33 (4): 533.
5. Piraccini B. M. and Alessandrini A. (2015). Onychomycosis: a review. *Journal ofFungi*, 1 (1): 30-43.
6. Vandeputte P., Ferrari S. and Coste A. T. (2011). Antifungal resistance and newstrategies to control fungal infections. *International Journal of Microbiology*: 2012
7. Robert R, Pihet M. Conventional methods for the diagnosis of dermatophytosis. Mycopathologia 2008;166(5-6):295–306
8. Clinical and Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard Second Edition. CLSI Document M38– A2. Wayne, PA:: CLSI;; 2008
9. Al-Janabi A. H. (2014). Dermatophytosis: causes, clinical features, signs and treatment. *Journal of Symptoms and Signs*, 3 (3): 200-
10. Kumar S. (2017). Clinico Microbial Identification and Characterization of Dermatophytes and Mrsa in Jaipur.
11. Khade A. S., Burute S. R., Deogude S. S., Jadhav P. and Raman and S. J. A (2018). Study of Clinical Profile of Dermatophytosis with a Changing Clinical Pattern at a Tertiary Care Centre.

Journal of Medical Science and Clinical Research, 6 (05):622-670.
12. Verma S. and Madhu R. (2017). The great Indian epidemic of superficialdermatophytosis: An appraisal. *Indian Journal of Dermatology*, 62 (3): 227.
13. Konda C., Surekha J. K., Jahnavi I., Madhuri D. S. and Nagamani K. (2017). Isolation and Identification of Dermatophytes in a Tertiary Care Hospital. *International Journal of Current Microbiology and Applied Sciences*, 6(2): 4088-4101.
14. Doddamani P.V., Harshan K.H., Kanta R.C., Gangane R., Sunil K.B. (2013) *People's Journal of Scientific Research*, 6:10-3.
15. Janagond A.B., Rajendrant T., Acharya S., Vithiya G.R.A., Charles J. (2016) *National Journal of Laboratory Medicine*, 5: MO29-MO32.
16. Hosthota A., Gowda T. and Manikonda R. (2018). Clinical profile and risk factors of dermatophytoses: a hospital based study. *International Journal of Research*, 4 (4): 508.
17. Singal A. and Khanna D. (2011). Onychomycosis: Diagnosis and management. *Indian Journal of Dermatology, Venereology and Leprology*, 77 (6): 659.
18. Poojary S, Miskeen A, Bagadia J, Jaiswal S, Uppuluri P. A study of in vitro antifungal susceptibility patterns of dermatophytic fungi at a tertiary care center in Western India. Indian J Dermatol 2019;64 (04):277–284
19. Singh A, Masih A, Khurana A, et al. High terbinafine resistance in Trichophyton interdigitale isolates in Delhi, India harbouring mutations in the squalene epoxidase gene. Mycoses 2018;61 (07):477–484
20. Altınbaş R, Özakkaş F, Barış A, Turan D, Şen S. In vitro susceptibility of seven antifungal agents against dermatophytes isolated in İstanbul. Turk J Med Sci 2018;48(03):615–619
21. Kulkarni SS, Bhakre JB, Damle AS. In vitro susceptibility testing of four antifungal drugs against fungal isolates in onychomycosis. Int J Res Med Sci 2018;6:2774–2780
22. Badali H, Mohammadi R, Mashedi O, de Hoog GS, Meis JF. In vitro susceptibility patterns of clinically important Trichophyton and Epidermophyton species against nine antifungal drugs. Mycoses 2015;58(05):303–307
23. Rudramurthy SM, Shankarnarayan SA, Dogra S, et al. Mutation in the squalene epoxidase gene of *Trichophyton interdigitale* and *Trichophyton rubrum* associated with allylamine resistance. Anti- microb Agents Chemother 2018;62(05):e02522–e17
24. Ansari S, Hedayati MT, Zomorodian K, et al. Molecular characteri- zation and invitro antifungal susceptibilityof 316 clinical isolates of dermatophytes in Iran. Mycopathologia 2016;181(1-2):89–95
25. Pathania S, Rudramurthy SM, Narang T, Saikia UN, Dogra S. A prospective study of the epidemiological and clinical patterns of recurrent dermatophytosis at a tertiary care hospital in India. Indian J Dermatol Venereol Leprol 2018;84(06):678–684
26. Budhiraja RK, Sharma S, Sharma S, et al. Antifungal susceptibility pattern of dermatomycosis in a tertiary care hospital of North India. Int J Res Dermatol 2018;4:240–245
27. Adimi P, Hashemi SJ, Mahmoudi M, et al. In-vitro activity of 10 antifungal agents against 320 dermatophyte strains using micro- dilution method in Tehran. Iran J Pharm Res 2013;12(03):537–545
28. Sahni K, Singh S, Dogra S. Newer topical treatments in skin and nail dermatophyte infections. Indian Dermatol Online J 2018;9 (03):149–158
29. Sahoo AK, Mahajan R. Management of tinea corporis, tinea cruris, and tinea pedis: a comprehensive review. Indian Dermatol Online J 2016;7(02):77–86