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IP Indian Journal of Clinical and Experimental Dermatology

Journal homepage: www.ijced.org/

Original Research Article

Clinico-microbiological study of dermatophytosis in a tertiary care center and emergence of multi-drug resistant dermatophyte *Trichophyton tonsurans* in the sub-population of West Bengaluru, Karnataka

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ARTICLE INFO

Article history:
Received 22-08-2023
Accepted 02-09-2023
Available online 17-10-2023

Keywords:
Dermatophytes
Trichophyton tonsurans
Multidrug resistant
Recalcitrant
Mycosis

ABSTRACT

Background: Antifungal susceptibility is an effective technique in managing clinical dermatophytosis due to an upsurge in resistant dermatophytes.
Objective: The intention of this research was to investigate the antifungal susceptibility profile of clinically significant dermatophytes in a tertiary care setting.
Materials and Methods: A total of 330 clinical samples were isolated from patients with superficial mycoses. Macroscopic characterization was done by examining the growth period, culture topography and synthesis of pigment on the reverse. The recovered strains were identified at the species level by ribosomal DNA (rDNA) internal transcribed spacer (ITS) regions sequencing. Antifungal susceptibility and MIC (Minimum Inhibitory Concentration) were determined using the Ezy MIC™ Strips (Himedia) on eight antifungals viz, Griseofulvin, Amphotericin B, Fluconazole, Itraconazole, Ketoconazole, Posaconazole, Terbinafine and Voriconazole,
Results: Out of 330 clinical isolates, 253 isolates were recovered and grown in culture, and identified by PCR sequencing. Tinea corporis was most predominant (65.15%) in the age group of 21-30 years. *Trichophyton rubrum* was the most abundant dermatophyte (47.83%), followed by *Trichophyton mentagrophytes (*30.43%) and *Trichophyton tonsurans* (19.76%). Posaconazole and voriconazole exhibited highest *in vitro a*ctivity followed by itraconazole and fluconazole. *Trichophyton mentagrophytes* yielded highest number of antifungal-resistant isolates (89.61%), followed by *T. rubrum* (86.78%) and *T. tonsurans* (76%). Posaconazole was found to be the most potent antifungal while amphotericin B was the least. *Trichophyton tonsurans* emerged as an important dermatophyte with significant resistant isolates.
Conclusion: Analysis of our data revealed distressing existence of multi-drug resistant dermatophytes in the sub-population and also a prominent shift in the prevalence from resistant *T. rubrum* to *T. mentagrophytes*. The emergence of a less prevalent dermatophyte in India, *Trichophyton tonsurans*, was witnessed in the study population.

1. Introduction

The Arthrodermataceae family of filamentous fungi includes the monophyletic group of dermatophytes, which are most recognized for inflicting superficial infections in both human and animals. They are the primary causative

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https://doi.org/10.18231/j.ijced.2023.025

agents of dermatophytoses, a type of superficial cutaneous mycosis that affects the skin, hair, and nails resulting in tinea pedis, tinea cruris, tinea capitis, or tinea corporis.[1] Although dermatophytes have a global distribution, some species are restricted to specific geographical regions of the world. The major species of dermatophytes occurring in Europe, the eastern Mediterranean region and South America are *Trichophyton rubrum, Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Epidermophyton floccosum* and *Microsporumcanis*.[2] Earlier studies have reported *T. rubrum* as the most common agent causing dermatophytosis in India followed by *T. verrucosum* and *T. interdigitale*.[3] However, there was a change in the dermatophytes pattern in India in the last 5 years with a rising prevalence of *T. mentagrophytes*, a zoophilic dermatophyte.[4]

The prevalence of resistant dermatophytoses has increased during the past several years which primarily manifest as tinea corporis and tinea cruris. Despite not being life-threatening, these diseases still make the victims physically uncomfortable. A rise in the incidence of dermatophytosis has been observed over the previous two decades, particularly in immunocompromised patients with organ transplantation, diabetes, AIDS and cancer.[5] To manage mycoses, there are already a fair variety of antifungal medications available in the market. However, several researchers have discovered that the fungus show tolerance or resistance to various medications, leading to the development of multidrug resistant (MDR) phenotypes.[6] All species of dermatophytes do not display the same pattern of susceptibility to different antifungal medications, indicating that antifungal resistance is a multifactorial process that is not completely understood. Also, the emergence of new species of dermatophytes in a sub-population and their treatment with appropriate antifungals are matters of serious concern. Hence, *in vitro* antifungal susceptibility testing may be useful for improving the management of the dermatophytosis.

This investigation was conducted to reveal the prevalence and antifungal susceptibility pattern of dermatophyte species recovered from superficial mycoses of human patients visiting the out-patient department of our tertiary care, referral hospital.

2. Materials and Methods

2.1. Ethical statement

The study was approved by the Institutional Ethics Committee of BGS Global Institute of Medical Sciences, Bengaluru, vide letter no. BGSGIMS/IEC/App/Dec/02 dated 14.12.2019.

2.2. Inclusion criteria

All patients reporting to the dermatology OPD clinically diagnosed with dermatophytosis.

2.3. Exclusion criteria

Pregnant patients and those on long term steroids and immunosup pressant drugs.

2.4. Chemicals

All antifungals (Amphotericin B, Fluconazole, Griseofulvin, Itraconazole, Ketoconazole, Posaconazole, Terbinafine and Voriconazole) were purchased from M/s Yarrow Chem, Mumbai. Fungal growth and selection media and, other solvents were procured from Himedia Laboratories, Mumbai.

2.5. Clinical fungal isolates

A total of 330 clinical fungal samples were isolated from patients with superficial mycoses reporting to the dermatology OPD between June 2020 and April 2021. In addition, 6 reference strains were used as controls: *Trichophyton rubrum* MTCC (Microbial Type Culture Collection) 296, *T. mentagrophytes* MTCC 7687, *T. tonsurans* MTCC 8475, *T. violaceum* ATCC 8376, *Epidermophyton floccosum* MTCC 7880 and *Microsporum gypseum* MTCC 9987.

2.6. Mycological identification and culture

The culture isolates were mycologically identified using macroscopic, microscopic, and molecular techniques. The samples of skin scales were subjected to direct microscopic examination using the wet mount method in 20% potassium hydroxide (KOH). To isolate dermatophytes specifically, the KOH positive isolates were grown in Sabouraud Cycloheximide Chloramphenicol agar (Himedia, India). Until good conidiation was attained, usually within 7–21 days, isolates were incubated in dark at 25–30 ^{0}C. Macroscopic characterization was done by examining the growth period, culture topography and synthesis of pigment on the reverse.

2.7. Molecular identification

By sequencing the ribosomal DNA (rDNA) internal transcribed spacer (ITS) sections, the culture-recovered strains were identified at the species level. After being grown in Sabouraud dextrose broth at 30°C for 7 days, fungus mycelia were recovered by filtration, powdered in a mortar in liquid nitrogen, and DNA was extracted using the DNeasy$^{©}$ Blood & Tissue Kit (Qiagen, GmBH). Integrity and quantitation of DNA was performed by determining the 260/280 ratio. The ITS rDNA region was amplified by using pan-fungal primers for ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). Sequencing was performed by the BigDye^{TM} Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher

Scientific, USA). The acquired sequences were compared with GenBank (NCBI).

2.8. *In vitro antifungal susceptibility testing*

Determination of antifungal sensitivity and MIC (Minimum Inhibitory Concentration) were done using Ezy MIC™ Strips (Himedia, Mumbai, India), as per the manufacturer's manual. The antifungals used for screening and their concentration range are - Amphotericin B (0.002-32 μg/ml), Ketoconazole (0.002-32 μg/ml), Itraconazole (0.002-32 μg/ml), Griseofulvin (0.002-32 μg/ml), Fluconazole (0.002-32 μg/ml), Posaconazole (0.002-32 μg/ml), Terbinafine (0.002-32 μg/ml) and Voriconazole (0.002-32 μg/ml).

2.9. *Statistical analysis*

Analysis of the data was done by SPSS (version 26.0). Comparison of MIC data was done using Student's t-test and the Mann-Whitney-Wilcoxon test. Values of $p \leq 0.05$ were considered as statistically significant.

3. Results

In patients with cutaneous fungal infections, direct microscopy (wet mount in 20% KOH) and culture (Sabouraud Cycloheximide Chloramphenicol agar) were used to confirm the occurrence of 330 incidences of dermatophytosis, including 153 males (46.36%) and 177 females (53.63%), the male to female ratio of occurrence being 0.86. The age range of the patients was 6 months to 90 years, with the most prevalent age group being between 21 and 30 (38.79%). The most common clinical presentation was found to be tinea corporis (65.15%), which was followed by tinea cruris (28.79%), tinea pedis (3.03%), tinea incognito (2.12%) and tinea manuum (0.91%) (Table 1, Figure 1).

Out of 330 clinical isolates, 253 isolates could be recovered and grown in culture. By sequencing the ribosomal DNA (rDNA) internal transcribed spacer (ITS) sections, they were identified at the species level. *Trichophyton rubrum* was the most abundant dermatophyte (n=121; 47.83%), followed by *Trichophyton mentagrophytes* (n=77; 30.43%), *Trichophyton tonsurans* (n=50; 19.76%), *Trichophyton violaceum* (n=2; 0.79%), *Epidermophyton floccosum* (n=2; 0.79%), and *Microsporum gypseum* (n=1; 0.40%; Figure 2). The majority of instances of the clinical manifestations of dermatophytosis, including tinea corporis, tinea cruris, tinea pedis, tinea incognito, and tinea manuum, were found to be caused by *Trichophyton rubrum*, followed by *T. mentagrophytes* and *T. tonsurans* (Table 2).

Table 3 displays the MIC50, MIC50 ranges, and geometric mean MIC values for each antifungal agent. Posaconazole and voriconazole exhibited highest *in vitro* activity against *T. tonsurans* (0.01 μg/ml), *E. flocossum* (0.002 μg/ml), *T. mentagrophytes* (2.36 μg/ml) and *M. gypseum* (0.002 μg/ml), followed by itraconazole against *T. rubrum* (0.04 μg/ml) and fluconazole against *T. violaceum* (0.17 μg/ml).

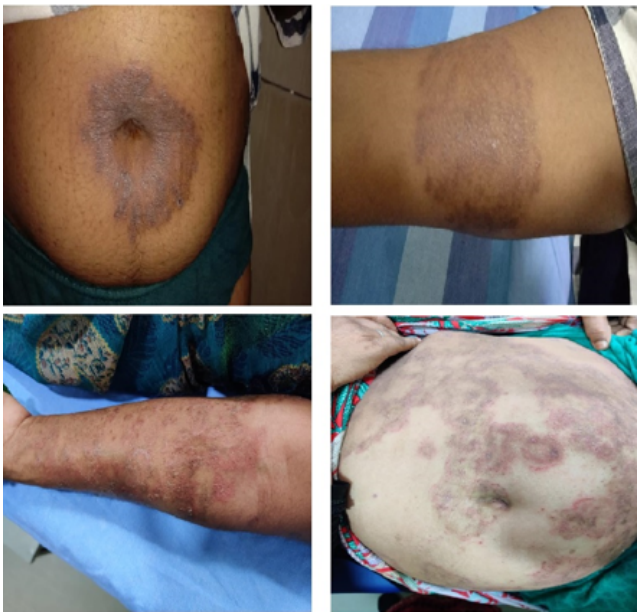

Fig. 1: Representative pictures of dermatophytic infections.

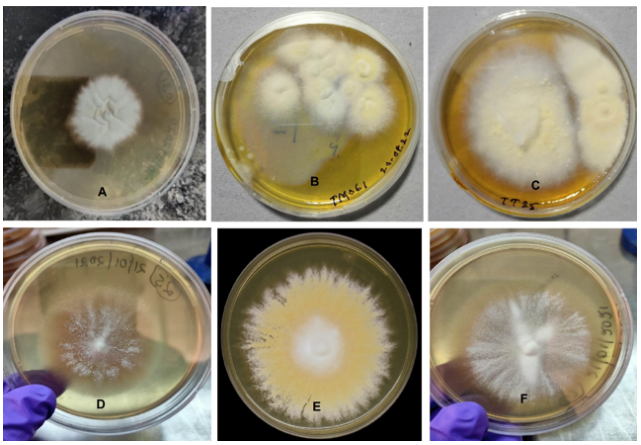

Fig. 2: Representative dermatophytes isolated from the samples and cultured on Sabouraud Cycloheximide Chloramphenicol agar. **A.** *Trichophyton rubrum*. **B.** *Trichophyton mentagrophytes*. **C.** *Trichophyton tonsurans*. **D.** *Epidermophyton floccosum*. **E.** *Microsporum gypseum*. **F.** *Trichophyton violaceum*

Interpretation of the MIC breakpoints was performed and the isolates are considered 'sensitive', 'intermediate' and 'resistant' to the concerned antifungal agent as per the interpretative criteria provided by CLSI guidelines. The dermatophytes species with highest number of resistant isolates was *Trichophyton mentagrophytes* (69/77; 89.61%), followed by *T. rubrum* (105/121; 86.78%) and *T. tonsurans*

Table 1: Correlation between type of dermatophytosis and range of age.

Clinical forms	Age groups									
	0-10 n (%)	11-20 n (%)	21-30 n (%)	31-40 n (%)	41-50 n (%)	51-60 n (%)	61-70 n (%)	71-80 n (%)	81-90 n (%)	Total
Tinea corporis	3 (1.4)	20 (9.30)	82 (38.14)	67 (31.16)	32 (14.88)	8 (3.72)	3 (1.4)	0 (0)	0 (0)	215 (65.15)
Tinea cruris	3 (3.16)	6 (6.32)	41 (43.16)	25 (26.32)	13 (13.68)	7 (7.37)	0 (0)	0 (0)	0 (0)	95 (28.79)
Tinea pedis	0 (0)	1 (10)	1 (10)	3 (30)	2 (20)	1 (10)	1 (10)	0 (0)	1 (10)	10 (3.03)
Tinea incognito	0 (0)	0 (0)	2 (28.57)	5 (71.43)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (2.12)
Tinea manuum	0 (0)	0 (0)	2 (66.67)	1 (33.33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.91)
Total	6	27	128	101	47	16	4	0	1	330 (100)

Table 2: Distribution of 253 dermatophyte species found in terms of frequency and clinical manifestations of dermatophytosis.

Species identified by ITS sequencing	Clinical forms of dermatophytosis					Total n (%)	Resistant isolates n (%)
	Tinea corporis n (%)	Tinea cruris n (%)	Tinea pedis n (%)	Tinea incognito n (%)	Tinea manuum n (%)		
T. rubrum	72 (59.5)	39 (32.23)	7 (5.79)	2 (1.65)	1 (0.83)	121 (47.83)	105 (86.78)
T. mentagrophytes	51 (66.23)	21 (27.27)	4 (5.19)	1 (1.3)	0 (0)	77 (30.43)	69 (89.61)
T. tonsurans	31 (62)	15 (30)	2 (4)	1 (2)	1 (2)	50 (19.76)	38 (76)
T. violaceum	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.79)	2 (100)
E. flocossum	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (0.79)	1 (50)
M. gypseum	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.4)	1 (100)
Total	157 (62.06)	75 (29.64)	13 (5.14)	6 (2.37)	2 (0.79)	253 (100)	216 (85.38)

(38/50; 76%). Isolates of *E. flocossum*, *M. gypseum* and *T. violaceum* were not significant in numbers (Table 2). The total resistant isolates obtained was 216 out of 253 total isolates yielding a resistance percentage of 85.37%.

Irrespective of the species, majority of the isolates were found to be resistant to amphotericin B (28.24%), followed by griseofulvin (19.91%), terbinafine (17.59%), ketoconazole (10.65%), voriconazole (9.72%), fluconazole (7.87%), itraconazole (8.63%) and posaconazole (1.39%) (Table 4).

4. Discussion

This study reveals the scenario of dermatophytoses in the West Bengaluru sub-population and, for the first time, describes the trend of dermatophyte species prevalence in this region. Of 330 cases of dermatophytosis, 253 could be recovered in cultured and identified to the species level.

Dermatophytosis was most common in people between the ages of 21 and 30 (38.79%). The increasing prevalence of dermatophytosis in this age range may be related to the population's involvement in outdoor activities like farming and manual work, which puts them at risk for infection through environmental exposure. Similar studies conducted substantiate our results.[7,8] The most prevalent clinical expression was tinea corporis, followed by tinea cruris. Our findings support those of earlier research conducted in India[7,9,10] and other parts of the world.[11–13]

Trichophyton rubrum still retains its position as the most abundant dermatophytes (121/253) as also reported by others,[3,14] followed by *Trichophyton mentagrophytes* (77/253), however, others reported a change in the dermatophytes pattern in India in the last 5 years with a rising prevalence of *T. mentagrophytes*, a zoophilic dermatophyte.[4,15] This dermatophyte outcompetes the pathogens that were previously found in India, particularly *T. rubrum*, and largely replaces them as the etiology of tinea cruris, tinea corporis, and tinea faciei.[16] Out of the total isolates, 105 of *T. rubrum* and 69 of *T. mentagrophytes* were found to be multi-drug resistant. In addition to that, for the first time, we report the emergence of the anthrophilic dermatophyte *Trichophyton tonsurans* causing infection in most patients (50/253) of this region after *T. rubrum* and *T. mentagrophytes*. Such abundance of *Trichophyton tonsurans* has never been reported before and is a trend of its kind in this region.[2–4,17–19]

For systemic therapy of dermatophytosis in India, the most frequently accessible antifungal medications are often itraconazole, fluconazole, terbinafine, and griseofulvin.[20] Posaconazole and voriconazole have the lowest MICs in all the examined strains in the

Table 3: Geometric mean of MICs, MIC ranges, and MIC_{50} values of antifungal agents obtained by susceptibility testing of 253 strains of dermatophytes.

MIC (μg/mL)				
Organism (no. of strains)	**Drugs**	**Range**	**MIC $_{50}$**	**Geometric mean**
T. rubrum (121)	Amphotericin B	0.008-32	12.78	2.73
	Fluconazole	0.016-64	7.52	0.24
	Griseofulvin	0.002-32	2.81	0.10
	Itraconazole	0.002-2	0.29	0.04
	Ketoconazole	0.002-32	5.51	0.05
	Posaconazole	0.002-8	0.68	0.01
	Terbinafine	0.002-32	2.69	0.02
	Voriconazole	0.002-32	4.14	0.12
T. mentagrophytes (77)	Amphotericin B	1-32	22.21	16.61
	Fluconazole	0.016-32	7.07	0.49
	Griseofulvin	0.002-32	16.03	0.57
	Itraconazole	0.002-32	4.80	0.11
	Ketoconazole	0.002-32	9.25	0.22
	Posaconazole	0.002-32	4.58	0.01
	Terbinafine	0.002-32	8.70	0.84
	Voriconazole	0.002-32	2.36	0.03
T. tonsurans (50)	Amphotericin B	0.38-32	12.73	4.59
	Fluconazole	0.016-256	74.80	0.73
	Griseofulvin	0.002-32	5.80	0.36
	Itraconazole	0.002-0.38	0.08	0.02
	Ketoconazole	0.002-3	0.85	0.15
	Posaconazole	0.002-0.016	0.01	0.00
	Terbinafine	0.006-1.5	0.53	0.08
	Voriconazole	0.02-0.38	0.10	0.03
T. violaceum (2)	Amphotericin B	12-32	22.00	19.60
	Fluconazole	0.016-0.32	0.17	0.07
	Griseofulvin	8-32	20.00	16.00
	Itraconazole	0.002-16	8.00	0.18
	Ketoconazole	0.008-8	4.00	0.25
	Posaconazole	0.002-1	0.50	0.04
	Terbinafine	0.02-4	2.01	0.28
	Voriconazole	0.002-1	0.50	0.04
E. flocossum (2)	Amphotericin B	0.38-32	24.00	22.63
	Fluconazole	0.016-256	22.00	19.60
	Griseofulvin	0.002-32	1.57	1.26
	Itraconazole	0.002-0.38	0.04	0.03
	Ketoconazole	0.38-32	28.00	27.71
	Posaconazole	0.002-0.016	0.01	0.00
	Terbinafine	0.006-1.5	0.01	0.00
	Voriconazole	0.02-0.38	0.04	0.04
M. gypseum (1)	Amphotericin B	0.38-32	-	-
	Fluconazole	0.016-256	-	-
	Griseofulvin	0.002-32	-	-
	Itraconazole	0.002-0.38	-	-
	Ketoconazole	0.38-32	-	-
	Posaconazole	0.002-0.016	-	-
	Terbinafine	0.38-32	-	-
	Voriconazole	0.002-1	-	-

Table 4: Dermatophyte isolates resistant to various antifungal agents.

Antifungal	Number of resistant isolates						
	T. rubrum	*T. mentagrophytes*	*T. tonsurans*	*T. violaceum*	*E. flocossum*	*M. gypseum*	Total n (%)
Amphotericin B	25	21	12	1	1	1	61 (24.24)
Fluconazole	9	4	3	1	0	0	17 (7.87)
Griseofulvin	23	11	9	0	0	0	43 (19.91)
Itraconazole	5	4	1	0	0	0	10 (4.63)
Ketoconazole	11	7	5	0	0	0	23 (10.65)
Posaconazole	2	1	0	0	0	0	3 (1.39)
Terbinafine	18	15	5	0	0	0	38 (17.59)
Voriconazole	12	6	3	0	0	0	21 (9.72)
Total	105	69	38	2	1	1	216

current investigation, with fluconazole and itraconazole serving as intermediates. Amphotericin B did not exert inhibitory effects. *Trichophyton mentagrophytes* was most resistant to antifungals followed by *Trichophyton rubrum* that was most resistant to amphotericin B followed by griseofulvin and terbinafine. Same pattern is observed with *T. mentagrophytes* and *T. tonsurans*. Furthermore, 38 out of 50 isolates of *T. tonsurans* were found to be multi-drug resistant. In this case, the isolates of *T. tonsurans* were found to be most resistant to fluconazole (MIC_{50} 74.80 μg/ml), followed by amphotericin B and griseofulvin (12.73 & 5.80 μg/ml respectively). However, posaconazole and voriconazole proved to be the over-all best effective antifungal agent against all species of dermatophytes, which creates a possibility of their usage to be explored in recalcitrant dermatophytoses. Despite the fact that there are alternative methods to carry out susceptibility testing, it is still unclear how clinical assessment of the MIC values or breakpoints will determine whether the tested agent is susceptible or resistant. Clinical breakpoint (CBP) play a significant function in the clinical context for better patient care.[21] Clinical breakpoint is impacted by numerous variables, including MIC distribution, antifungal PK/PD data, and most significantly, the severity of the disease. It is challenging to determine the CBP for a specific species due to the dearth of data on clinical outcomes with antifungal susceptibility data. Consequently, in such circumstances, the epidemiological cutoff value (ECV) for each particular species and antifungal drug may be derived.

Multi-factorial factors, including host-derived, are involved in resistance to antifungal medications viz, personal hygiene, discontinuation of treatment regimen, irrational use of over-the-counter antifungal-corticosteroid creams, ineffective stratum corneum penetration,[20] to pathogen-derived viz, adaptive stress reactions due to the stress induced by antifungal and cytotoxic drugs usage in sub-inhibitory concentrations, up-regulation of cellular detoxification-related genes, cytosolic efflux of drug, signaling pathways, overexpression of the ATP-binding cassette transporter gene causing cellular efflux of antifungals and mutations.[21] Our findings call for additional research to understand the potential causes of antifungal drug resistance of *Trichophyton tonsurans*.

5. Conclusion

The study revealed significant involvement of multi-drug resistant dermatophytes in recalcitrant superficial skin infection in this particular sub-population. A shift in the prevalence from resistant *T. rubrum* to *T. mentagrophytes* has been witnessed that requires clinical attention. A prominent emergence of the less popular *Trichophyton tonsurans* along with significant measure of multi-drug resistant isolates has raised a concern, and opened wide options to conduct further research to circumvent the resistance.

6. Authors' Contribution

All the authors made significant contribution towards the conceptualization, investigation, data curation of the research work and, writing, review and editing of the article.

7. Informed Consent

Informed Consent Forms (ICFs) were obtained from individual patient before collection of samples.

8. Source of Funding

The study was funded by the department of Advanced Research, Rajiv Gandhi University of Health Sciences (RGUHS), Bengaluru, India, vide grant no. RGU:RGU/ADV-RES/BR-19/2019-20 dated 10.12.2019.

9. Conflict of Interest

The authors have no conflict of interest to declare.

10. Acknowledgement

The authors thank the department of Advanced Research, Rajiv Gandhi University of Health Sciences (RGUHS), Bengaluru, India, for funding the study. The authors express their deep sense of gratitude to the Dean cum Principal, Dr. M.E. Mohan for extending the facilities and infrastructure for conducting the experiments. The authors also thank the Institutional Ethics Committee for approving the study.

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Cite this article: Shantharaju SA, Samaddar S, Kumaraswamy B, Okade R, Koneri R, Joshua L. Clinico-microbiological study of dermatophytosis in a tertiary care center and emergence of multi-drug resistant dermatophyte *Trichophyton tonsurans* in the sub-population of West Bengaluru, Karnataka. *IP Indian J Clin Exp Dermatol* 2023;9(3):135-141.