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A Cross-Sectional Study on Recalcitrant Dermatophytosis in a Tertiary Care Centre in Thanjavur medical college: Clinical Profile, Mycological Patterns, and Antifungal Susceptibility.

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ABSTRACT

Background: Dermatophytosis has emerged as a major superficial fungal infection in India, with increasing cases showing recurrence, chronicity, or resistance termed recalcitrant dermatophytosis. This study was undertaken to evaluate clinical patterns, isolate dermatophytes, and determine antifungal susceptibility among patients presenting with chronic and recurrent dermatophytic infections. **Methods:** A cross-sectional study was conducted over one year at the Department of Microbiology, Thanjavur Medical College. Sixty clinically suspected patients with chronic, recurrent, or persistent dermatophytosis were enrolled. Specimen including skin scrapings, hair and nail clippings were subjected to direct KOH examination, culture on Sabouraud's Dextrose Agar and DTM and LPCB mount for identification Antifungal susceptibility testing (AFST) was performed using CLSI M38-A2 microbroth dilution for fluconazole, ketoconazole, griseofulvin, and terbinafine. **Results:** Dermatophytosis was most common among males (56.7%) and individuals aged 21–40 years. Among the isolates Trichophyton mentagrophytes predominanted species (40%), followed by Trichophyton rubrum (33.3%). Among antifungal agents, terbinafine showed the lowest MIC values ($MIC_{90} \leq 0.25$ µg/mL), while fluconazole exhibited the highest resistance ($MIC_{90} \geq 2$ µg/mL). A significant number of isolates demonstrated reduced susceptibility to fluconazole and griseofulvin. **Conclusion:** Recalcitrant dermatophytosis poses diagnostic and therapeutic challenges. The predominance of T. mentagrophytes and rising antifungal resistance especially to fluconazole necessitate species-level identification and regular susceptibility testing. Terbinafine remains the most effective oral antifungal agent. Rational use of antifungals and public awareness are critical in controlling this epidemic.

1. INTRODUCTION:

Dermatophytosis, commonly referred to as ringworm or tinea, is a superficial fungal infection affecting keratinized tissues including skin, hair, and nails. The infection is primarily caused by dermatophytes belonging to the genera Trichophyton, Microsporum, and Epidermophyton. In recent years, a notable increase in chronic, recurrent, and treatment-resistant infections has le d to the emergence of the clinical entity termed recalcitrant dermatophytosis [1] (Kaur et al., 2020).

The global prevalence of superficial fungal infections is estimated to be around 20–25% [4](Havlickova et al., 2008), with South Asia, particularly India, reporting a surge in chronic and relapsing dermatophytic infections over the past decade[5] (Agarwal et al., 2021). Factors such as widespread use of over-the-counter topical corticosteroid-antifungal combinations, inappropriate dosing and duration of systemic

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antifungal therapy, hot and humid climate, overcrowding, poor hygiene, and emerging antifungal resistance have been implicated in this resurgence[6,9] (Kumar et al., 2020; Verma & Madhu, 2017).

Recalcitrant dermatophytosis includes infections that are persistent despite adequate therapy, recurrent within four weeks of treatment cessation, or show microbiological resistance[6] (Kumar et al., 2019). Clinically, these infections present as extensive, itchy, and inflamed lesions and may involve multiple anatomical sites, frequently affecting young adults and males due to greater environmental exposure and sweating.

Traditionally, Trichophyton rubrum was considered the predominant etiological agent in India. However, recent studies report a shift in epidemiology, with Trichophyton mentagrophytes complex emerging as the leading pathogen and exhibiting increased resistance to commonly used antifungals such as fluconazole and griseofulvin [8,3](Singh et al., 2020; Dogra et al., 2019).

Accurate laboratory diagnosis, species identification, and antifungal susceptibility testing (AFST) are crucial in managing recalcitrant cases. The Clinical and Laboratory Standards Institute (CLSI) M38-A2 protocol remains the reference method for AFST in filamentous fungi (CLSI, 2008)[2]. Terbinafine and itraconazole continue to show efficacy, but sporadic resistance is emerging[7] (Martinez-Rossi et al., 2021).

This study was conducted and investigate the clinical and mycological spectrum of recalcitrant dermatophytosis in a tertiary care centre in Tamil Nadu, and to evaluate in vitro antifungal susceptibility patterns of the isolated dermatophytes, particularly in the context of increasing therapeutic failures.

MATERIALS AND METHODS:

Study Design and Setting: This analytical cross-sectional study was conducted over a period of 12 months (July 2020 to June 2021) in the Department of Microbiology in collaboration with the Department of Dermatology, Thanjavur Medical College and Hospital, Tamil Nadu, a tertiary care teaching hospital in South India.

Ethical Clearance: The study was approved by the Institutional Ethical Committee of Thanjavur Medical College (Approval dated 7.1.2019). written informed consent was obtained from all participating patients.

Study Population: The study included 60 patients clinically diagnosed with superficial dermatophytosis who presented with chronic, recurrent, or treatment-unresponsive lesions. Detailed clinical information, including age, sex, socioeconomic status, occupation, site of infection, prior treatment history, and co-morbidities was collected.

Inclusion Criteria:

- Patients with newly diagnosed or chronic dermatophytosis (skin, hair, or nails)
- Immunocompromised patients
- Recurrent cases within 4 weeks of completing antifungal therapy

Exclusion Criteria:

- Non-dermatophyte fungal infections (e.g., Malassezia, Candida)
- Drug-induced or eczematous skin lesions

Specimen Collection: Depending on clinical presentation, the following samples were collected under aseptic precautions:

- **Skin scrapings:** from active margins of lesions
- **Hair:** plucked from affected scalp areas including the root
- **Nail clippings:** from dystrophic, thickened, or discoloured nails

Microscopic Examination: Direct microscopy was performed using 10% KOH for skin and hair and 20% KOH for nails. Specimens were examined under low and high power to detect fungal elements such as hyaline septate hyphae and arthroconidia.

Culture and Identification: All specimens were cultured in duplicate on Sabouraud Dextrose Agar (SDA) with gentamicin and cycloheximide and SDA with gentamicin alone Plates were incubated at 27–30°C and monitored for 4 weeks. Dermatophytes were identified based on: Colony morphology, Lactophenol cotton blue (LPCB) mount, Dermatophyte Test Medium (DTM, Riddle's slide culture technique, Urease test on Christensen's medium,In vitro hair perforation test and Antifungal Susceptibility Testing (AFST).

AFST was performed using the CLSI M38-A2 broth microdilution method. The following antifungal agents were tested:

- Terbinafine (0.001 – 0.5 µg/mL)
- Griseofulvin (0.031 – 16 µg/mL)
- Ketoconazole (0.031 – 16 µg/mL)
- Fluconazole (0.125 – 64 µg/mL)

RPMI-1640 medium buffered with MOPS was used as the test medium. Inoculum suspensions were prepared at a density of 1–3 × 10^3 CFU/mL using the

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aid of a hemocytometer. Following incubation at 28°C, the plates were visually assessed on day 4
MIC values were defined as the lowest concentration of the antifungal agent that produced ≥80% inhibition of growth relative to the drug-free control well. Interpretation was done according to established breakpoints for dermatophytes (CLSI, 2008).

RESULTS:

An over-all of 60 patients clinically diagnosed with recalcitrant dermatophytosis were included in the study. The majority of the study participants were males (56.7%), with females accounting for 43.3%. Most patients belonged to the age group of 21 to 40 years, comprising 56.6% of the total population, followed by those aged 41 to 50 years (21.7%). Dermatophytosis was more often observed among individuals from lower socioeconomic backgrounds (63.3%) and was especially common among agricultural workers and homemakers, each representing 26.7% of the study group. Students accounted for 25% of cases.

Clinically, the most common form of presentation was Tinea corporis, observed in 78.3% of cases. Other clinical types included Tinea cruris (6.7%), Tinea barbae (5%), Tinea capitis (5%), Tinea unguinum (3.3%), and Tinea pedis (1.7%).

With respect to specimen collection, 91.6% were skin scrapings, 5% were hair samples, and 3.4% were nail clippings. Direct microscopic examination using KOH mount showed positivity in 50% of skin specimens, while all hair and nail samples were KOH negative. Fungal culture yielded growth in 31 out of 60 samples (51.6%). Among these, 30 were from skin scrapings and one from a hair sample. No fungal growth was attained from nail samples.

The predominant fungal isolate was Trichophyton rubrum, accounting for 58.6% of culture-positive cases, followed by Trichophyton mentagrophytes (27.6%), Epidermophyton floccosum (10.3%), and Microsporum gypseum (3.4%).

Antifungal susceptibility testing of the 29 dermatophyte isolates revealed terbinafine to be the most effective agent, with all isolates demonstrating sensitivity and MIC90 values ≤ 0.06 µg/mL. In contrast, fluconazole showed the highest resistance, with 37.9% of isolates showing MICs ≥ 2 µg/mL. Resistance to griseofulvin was observed in 24.1% of isolates, and ketoconazole showed resistance in 10.3% of cases.

Among the two most common isolates:

- Trichophyton rubrum showed high MICs for fluconazole (MIC90 = 16 µg/mL) but remained highly susceptible to terbinafine (MIC90 = 0.06 µg/mL).
- Trichophyton mentagrophytes also demonstrated sensitivity to terbinafine but had moderate MIC values for fluconazole and griseofulvin.

The findings suggest that terbinafine remains the most effective oral antifungal, while resistance to fluconazole is significantly high, highlighting the need for targeted antifungal therapy guided by susceptibility profiles.

Correlation Between Direct Microscopy (KOH Examination) and Culture Results with Respect to Clinical Diagnosis Observed in the Study.

S. No	Clinical diagnosis	KOH and culture positive (N= 27)		KOH positive culture negative (N=3)		KOH negative and culture positive (N=4)		KOH and Culture negative (N=26)		P value
		n	%	n	%	n	%	n	%	
1	Tinea barbae	1	3.7	0	0	1	25	1	3.8	0.347 (NS)
2	Tinea Captis	1	3.7	0	0	1	25	1	3.8	
3	Tinea Corporis	22	81.5	3	100	2	50	20	76.9	
4	Tinea cruris	0	0	0	0	0	0	4	15.4	
5	Tinea pedis	1	3.7	0	0	0	0	0	0	
6	Tinea unguim	2	7.4	0	0	0	0	0	0	

Out of 31 isolates 27 were both KOH and culture positive and 4 were KOH negative and culture positive. Out of 31 cases T.corporis accounted for 24 (77.4%) cases followed by Tinea Captis 2 (6.45%) cases and Tinea barbae 2(6.45%), Tinea unguim (6.45%), and Tinea pedis 1(3.22%).

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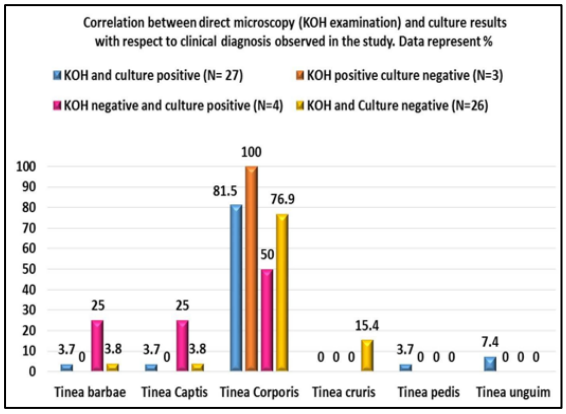

DISCUSSION

Dermatophytosis continues to pose a significant public health concern, particularly in tropical regions like India. The emergence of recalcitrant dermatophytosis, characterized by chronic, recurrent, or treatment-resistant lesions, has added to the complexity of clinical management. This study highlights the clinical profile, mycological spectrum, and antifungal susceptibility of dermatophytes among affected individuals attending a tertiary care hospital in Tamil Nadu.

In the present study, young adults aged 21–40 years reported for the majority of recalcitrant dermatophytosis cases (56.6%). This demographic is frequently reported as the most affected group in several Indian studies due to their active lifestyle, occupational exposure, and increased physical activity that predisposes them to excessive sweating and friction (Agarwal et al., 2021; Kaur et al., 2020)[1,5]. Kaur et al. (2020) similarly reported 54.2% of cases in this age range, reinforcing our findings[1].

A slight male preponderance (56.7%) was also observed, comparable to earlier studies such as Singh et al. (2020)[8], who proved a male-to-female ratio of approximately 1.3:1. The increased incidence in males may be related to environmental exposure, tight clothing, poor ventilation, and use of occlusive footwear (Verma & Madhu, 2017)[9].

The disease burden was significantly higher among individuals from lower socioeconomic strata, consistent with previous observations (Dogra et al., 2019; Pande & Mishra, 2019)[3,10]. Overcrowded living conditions, limited awareness of personal hygiene, and delayed healthcare-seeking behaviour contribute to the persistence and chronicity of dermatophytoses in these populations.

Tinea corporis was the most common clinical presentation (78.3%) in our cohort. Similar findings were reported by Bishnoi et al. (2018), who observed 74.6% of patients presenting with tinea corporis[11]. The high frequency of tinea corporis and cruris in current Indian studies reflects both their chronicity and ease of transmission, particularly in hot, sticky environments.

Culture positivity in our study was 51.6%, a figure aligned with Kaur et al. (2020), who noted a positivity rate of 53.8%[1]. However, studies like Pande et al. (2019) reported lower rates (30–40%)[10], possibly due to differences in sample types, techniques, and media used. While KOH mount remains a rapid diagnostic tool, its sensitivity was limited, particularly in hair and nail samples, highlighting the essential role of culture for definitive diagnosis.

The predominant isolate was *Trichophyton rubrum* (58.6%), followed by *T. mentagrophytes* (27.6%). This mirrors historical global trends (Nenoff et al., 2014)[12]. Still, several recent Indian studies, similar as those by Rudramurthy et al. (2018) and Singh et al. (2020), have observed a significant epidemiological shift, with *T. mentagrophytes*, especially genotype VIII, overtaking *T. rubrum* in chronic and inflammatory infections [13,8].

Terbinafine showed the highest efficacy in our study (100% susceptibility), with an MIC90 ≤ 0.06 µg/mL. Similar results were observed in studies by Shankarnarayan et al. (2019) and Monod et al. (2021)[13]. The fungicidal mechanism of terbinafine and its high tissue penetration makes it highly effective, particularly in recalcitrant infections.

Conversely, resistance to fluconazole (37.9%) and griseofulvin (24.1%) was notable. Fluconazole resistance has been increasingly reported in Indian settings, with Martinez-Rossi et al. (2021)[7] attributing it to the indiscriminate use of over-the-counter formulations. In a study by Singh et al. (2020)[8], fluconazole resistance was observed in nearly 40% of isolates. Resistance to griseofulvin, although less studied, may stem from prolonged treatment durations and its fungistatic nature.

Ketoconazole resistance was relatively low (10.3% in our study), but its hepatotoxic potential limits its clinical utility. With increasing reports of terbinafine resistance due to mutations in the squalene epoxidase gene (Rudramurthy et al., 2018)[13], continued surveillance using standardized methods such as CLSI M38-A2 becomes imperative.

LIMITATIONS:

This study was limited by a modest sample size, impacted by the COVID-19 pandemic. Additionally, genotypic analysis of *T. mentagrophytes* strains was not performed, which could have provided deeper insights into emerging resistant strains. Future multicentric studies with molecular epidemiology and longer follow-up periods are essential.

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CONCLUSION:

Our findings underscore the shifting epidemiology and emerging resistance patterns in dermatophytosis, particularly in recalcitrant cases. *T. rubrum* and *T. mentagrophytes* remain the leading etiological agents, with terbinafine as the most effective therapeutic option. Rising resistance to fluconazole and griseofulvin demands vigilant antifungal stewardship and regular surveillance using standardized AFST. A multifaceted approach involving rational drug use, public health education, and diagnostic standardization is essential to combat this emerging epidemic.

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