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Antifungal Susceptibility Profile of Trichophyton species with Comparative Evaluation of Conventional Antifungal Agents and Efinaconazole

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ABSTRACT
Dermatophytes, responsible for widespread skin infections globally, are keratinophilic fungi that infect the skin, hair, and nails. Despite being often considered mild, dermatophytosis can lead to significant physiological and psychosocial consequences, particularly in cases of chronicity, stigma, and treatment costs. While conventional antifungal agents such as itraconazole, terbinafine, and ciclopirox are used to treat these infections, their efficacy is increasingly compromised by factors such as poor drug penetration, interactions, and, more critically, the emergence of antifungal resistance. Resistance, particularly to azoles and terbinafine, is becoming a major concern, especially in regions with hot, humid climates like India. Recent studies indicate a growing prevalence of resistant strains of dermatophytes, with resistance rates reaching up to 71% for terbinafine in certain regions. Newer antifungal agents, such as efinaconazole, have shown promising results, outperforming traditional treatments in terms of efficacy against dermatophytes. This study evaluates the effectiveness of efinaconazole in comparison to conventional antifungal agents, aiming to guide clinicians in selecting the most appropriate treatment for chronic and resistant dermatophytosis cases. Antifungal susceptibility testing (AFST) is emphasized as a crucial tool for optimizing treatment strategies and addressing the rising challenge of antifungal resistance in dermatophyte infections.

INTRODUCTION:

Dermatophytes cause widespread and persistent skin infections affecting people globally[1]. These keratinophilic fungi, which are commonly referred as "ringworm" or "tinea," infiltrate the dead keratinised layers of skin, hair, and nail. They are members of the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*[2]. While often considered trivial, dermatophytosis can have significant physiological effects due to stigma, cosmetic involvement, chronicity, and the cost of treatment[3].

A variety of antifungal agents, including itraconazole, terbinafine, amorolfine, and ciclopirox, have been used to treat superficial fungal skin infections caused by dermatophytes. However, the efficacy of these conventional drugs is

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sometimes hampered by issues such as drug interactions and low permeation[4]. More critically, there is growing evidence of fungi developing resistance to certain antifungal drugs, leading to therapeutic failures. This phenomenon of acquired antifungal resistance is on rise, especially with increased and prolonged use of antifungal medications[5]. Reports indicate a significant rise in dermatophytosis cases with chronicity, recalcitrant disease, atypical presentations, frequent relapses, and treatment failures[6].

A concerning pattern of dermatophyte resistance to antifungal agents has been noted in recent years, which has led to an increase in chronic and recalcitrant cases of dermatophytosis, especially in countries with hot and humid climates like India[7]. Azole resistance in dermatophytes has been increasingly associated with the widespread use of azole antifungals, unlike allylamines such as terbinafine and naftifine; however, documented cases of this resistance are only beginning to emerge. According to reports, up to 19% of dermatophytes globally exhibit azole resistance[8]. Fluconazole has been found to be the least effective drug; revealed resistance rates of up to 61.33% in Western Rajasthan[9]. It's concerning to note that terbinafine, a first-line treatment, has also seen a rise in resistance, particularly in India. In a multicentric study conducted in India, about 71% of the dermatophyte isolates were found to be terbinafine resistant[10]. Griseofulvin, the first available antifungal, is now rarely used due to the development of safer alternatives. While some studies report low resistance rates (5.33%), treatment failures have been documented since the 1960s[6]. In numerous studies, voriconazole has consistently demonstrated very low or no resistance, frequently as a result of its limited availability or use in particular areas[6]. In order to treat dermatophyte infections, especially onychomycosis, the FDA has approved efinaconazole, a novel antifungal agent, in 2014. Efinaconazole has shown significant antifungal activity against dermatophytic fungi, often outperforming conventional agents like terbinafine, ciclopirox, itraconazole, and amorolfine. When compared to other conventional drugs, efinaconazole has demonstrated encouraging outcomes with comparatively low minimum inhibitory concentration (MICs) for *T. rubrum* and *T. mentagrophytes* [4].

Measuring the relative effectiveness of antifungal agents is a crucial tool in the clinical management of dermatophytosis. In this context, antifungal susceptibility testing (AFST) for dermatophytes has become an urgent need.[11]. Hence this study was aimed to assess the effectiveness of efinaconazole in comparison to conventional antifungal drugs used for the treatment of dermatophytosis. This will assist the clinician in selecting the appropriate antifungal agent for treating chronic and recalcitrant dermatophytosis.

MATERIALS AND METHODS:

Sample collection: All the samples (skin, hair, and nail) which were KOH and culture positive collected over a period of 12 months (From 1st June 2024 to 31st May 2025) were considered for this study, after obtaining Institute ethics approval (CSP-MED/23/SEP/93/220). The samples were collected from patients suspected to have dermatophytosis, and sent to the microbiology laboratory on a sterile thick black chart paper.

Direct microscopy: The samples were subjected to 10% potassium hydroxide (KOH) for skin and hair samples and 40% KOH for nail samples.

Culture: All samples were cultured on Sabouraud Dextrose Agar (SDA), Dermatophyte test medium and Oatmeal agar with antibiotics and cycloheximide, and incubated at 25ºC and 37ºC for 4 weeks. The colony morphology and the pigmentation were observed in SDA. Lactophenol cotton blue (LPCB) mount was done from colonies. The genus and species level identification of the isolates using microscopic morphology was done as per de Hoog et. al.,[12].

Other tests like urea hydrolysis and hair perforation were also performed, which aids in the species level identification.

ANTI-FUNGAL SUSCEPTIBILITY TESTING:

The colonies from Oatmeal agar were used for testing the antifungal susceptibility. The Antifungal Susceptibility Testing was performed using broth micro-dilution method, based on the Clinical and Laboratory Standards Institute (CLSI) guidelines M27-A3. Six antifungal agents namely Fluconazole (32 - 0.125µg/mL), Itraconazole (16 – 0.0625µg/mL), Voriconazole (16 – 0.0625µg/mL), Terbinafine (16 – 0.0625µg/mL), Griseofulvin (16µg/mL – 0.0625µg/mL) and Efinaconazole (16 – 0.0625µg/mL), (Sigma Chemical Corporation, St. Louis, MO, USA) were used. *T. mentagrophytes* ATCC 4439 was used as a quality control strain.

The microtiter plates were incubated at 25°C for five days, or until visible growth was observed in the growth control well. All tests were performed in triplicate, and the mean MIC for each drug was calculated for each isolate. An 80% reduction in growth, compared to the growth control well, was considered as the endpoint for determining the MIC.

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RESULTS:

In this study, 97 KOH and Culture positive samples were collected from skin scraping (n-92), nail (n-3), and hair (n-2).

The age distribution revealed that the majority of patients (71 cases) were between 21 and 50 years old (Fig 1), with a median age of 38 years. Among the total, 52 (53.6%) were male and 45 (46.4%) were female, resulting in a male-to-female ratio of 1.15:1. This indicates a slight predominance of males over females in the study population.

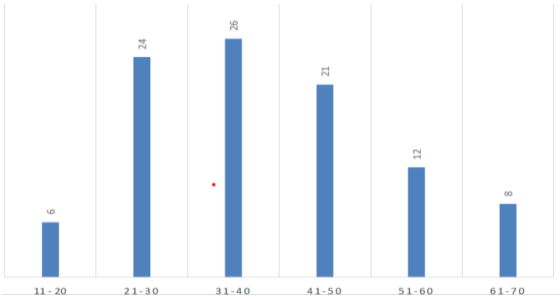

Figure1: Distribution of dermatophytosis cases in relation to age group

Direct microscopy of all the 97 samples showed the presence of narrow, hyaline, septate (Fig 2). It helps in differentiation between yeast and mould causing infections.

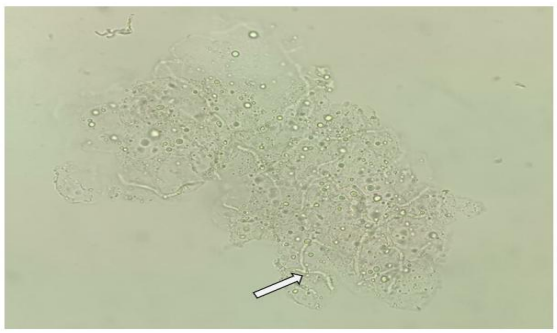

Figure -2: 10% KOH mount showing narrow septate hyphae (40X)

Culture results showed that the majority of isolates were *Trichophyton mentagrophytes* species complex (n = 90), characterized by powdery to floccose colonies, cream-colour to yellowish-buff (Fig. 3a) and on reverse yellow pigmentation (Fig. 3b). In contrast, *Trichophyton rubrum* (n = 7) produced fluffy to cottony, white colonies, (Fig. 4a) with a characteristic wine-red pigmentation on the reverse (Fig. 4b). Lactophenol cotton blue staining of *T. mentagrophytes* species complex revealed cigar-shaped macroconidia with 3-8 celled, smooth and thin walled, abundant microconidia, arranged in clusters and spiral hyphae (Fig. 3c). In *T. rubrum,* pencil-shaped macroconidia and tear-drop–shaped microconidia attached to their bases along the hyphae in rows (bird on wire appearance) were observed (Fig. 4c).

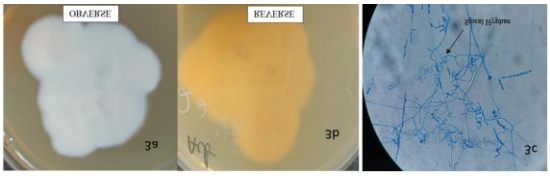

Figure 3(a,b): *T.mentagrophytes* species complex culture on SDA medium showing white, floccose colonies with yellow on reverse

Fig 3 c: LPCB mount showing microconidia in clusters and spiral hyphae (40X)

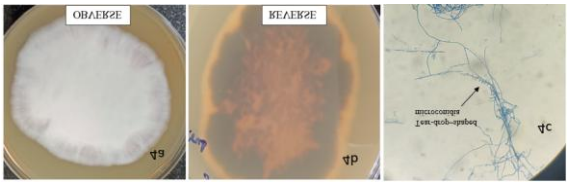

Figure 4 (a,b): *T.rubrum* culture on SDA showing fluffy colonies (Fig. 4a) with a wine-red pigmentation on the reverse

Fig 4 c: LPCB mount showing tear-drop–shaped microconidia arranged in rows on either side oh hyphae (40X)

The antifungal susceptibility profile for the *Trichophyton* species isolated for the six antifungal agents tested is shown in Table 1.

Table 1: In-vitro susceptibility pattern of *Trichophyton* species (n=97) against the antifungal agents.

S.No	DRUG	Geometric mean (µg/mL)	Standard Deviation	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
1	Fluconazole	13.57	12.33	16	32
2	Itraconazole	0.641	2.187	0.5	4
3	Voriconazole	0.076	0.043	0.0625	0.125
4	Griseofulvin	0.150	2.27	0.0625	0.5
5	Terbinafine	0.229	4.64	0.06	8
6	Efinaconazole	0.0643	0.0124	0.0625	0.0625

Fluconazole had the highest geometric mean MIC (13.57 µg/mL), with high MIC_{50} and MIC_{90} values of 16 µg/mL and 32 µg/mL, respectively, indicating reduced efficacy of this drug for treating dermatophytic infections. Itraconazole had a much lower geometric mean (0.641 µg/mL) with wide variation (SD = 2.187), and low MIC_{50} of 0.5 µg/mL, but slightly higher MIC_{90} value of 4 µg/mL, indicating its reduced efficacy against *Trichophyton* species. Voriconazole exhibited the highest antifungal activity, with a very low geometric mean MIC of 0.076 µg/mL and a narrow MIC distribution, as reflected by MIC_{50} and MIC_{90} values of 0.0625 µg/mL and 0.125 µg/mL, respectively.

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Griseofulvin and terbinafine demonstrated moderate activity; although both had low MIC_{50} values (0.0625 µg/mL and 0.06 µg/mL, respectively), their elevated MIC_{90} values (0.5 µg/mL for griseofulvin and 8 µg/mL for terbinafine) and high standard deviations indicate variable susceptibility among isolates. Efinaconazole showed consistently strong activity, with the least variability (SD = 0.0124) and identical MIC_{50} and MIC_{90} values of 0.0625 µg/mL.

In this study, among the 97 dermatophyte isolates tested, 90 were *Trichophyton mentagrophytes* species complex and 7 were *T. rubrum*. The species wise difference in the antifungal susceptibility pattern is shown in the Table 2.

Table 2: High MIC percentage in *Trichophyton* species: *Trichophyton mentagrophytes* species complex Vs *Trichophyton rubrum*

ORGANISM	*Trichophyton mentagrophytes* species complex n=90				*Trichophyton rubrum*n=7			
DRUG	LOW MIC		HIGH MIC		LOW MIC		HIGH MIC	
	n	%	N	%	N	%	N	%
Fluconazole	2	2.2	88	97.7	1	14.2	6	85.7
Itraconazole	64	71.1	26	28.8	5	71.4	2	28.5
Voriconazole	90	100	0	0	7	100	0	0
Griseofulvin	86	95.6	4	4.4	7	100	0	0
Terbinafine	68	75.6	22	24.4	7	100	0	0
Efinaconazole	90	100	0	0	7	100	0	0

Among the antifungal agents evaluated, fluconazole exhibited the lowest efficacy, with high MIC values observed in 93.3% of *T. mentagrophytes* species complex and 71.4% of *T. rubrum*. In contrast, itraconazole demonstrated moderate activity, with nearly half of the isolates from both species showing low MICs. For griseofulvin, 4.4% of *T. mentagrophytes* species complex isolates showed high MICs, whereas terbinafine resistance was more prominent, with approximately 25% exhibiting high MICs. All *T. rubrum* isolates remained susceptible to both griseofulvin and terbinafine. Notably, all isolates from both species were fully susceptible to voriconazole and efinaconazole, indicating their superior antifungal efficacy.

DISCUSSION:

Superficial dermatophytosis has become a growing global concern, affecting approximately 20-25% of the world's population[13]. It poses significant clinical, emotional, and financial burden on those affected. Misuse of over-the-counter antifungal-steroid creams has contributed to a rise in recalcitrant and recurrent dermatophytosis cases. This along with augmented antifungal resistance has also become a major challenge. Antifungal susceptibility testing is crucial in detecting resistance patterns and guiding suitable treatment.

In the current study, the male to female ratio of patients suffering from superficial dermatophytosis was found to be 1.15:1, where it has been observed that the males are affected slightly higher than the females. In the year 2018, a study conducted by Hazarika *et. al.,* in a tertiary care centre in Assam revealed the male and female ratio to be 1.5:1 [14]. In a study conducted by Patro *et al.*, from Odisha in the year 2019, females were commonly affected than males with the male-female ratio being 0.88:1[15]. The slight preponderance in males may be attributed to differences in occupational exposure, hygiene practices and healthcare seeking behaviour.

The commonest age group affected was found to be aged 31-40 years followed by 21-30 and 41-50 years of age. The median age was found to be 38 years. In a study conducted by Patro *et al.,* from Assam the most common age group was 18-40 years of age (15). Similar to our study, the commonest age group affected was 31-40 years followed by 21-30 years of age was found in a study by Maulingar *et al.,* in the year 2014[16]. Factors such as lifestyle, occupational exposure and tendency to seek medical care contributes to the predominance of dermatophyte infections among the middle-aged individuals.

Fluconazole remains one of the most prescribed antifungals for tinea infections, but its overuse has led to increasing resistance, particularly among *Trichophyton* isolates. In our study, fluconazole exhibited a high geometric mean MIC of 13.57µg/mL, with MIC_{50} and MIC_{90} values at 16µg/mL and 32µg/mL, respectively, reflecting reduced susceptibility. Similar findings have been reported in previous studies, highlighting regional variations in resistance. Resistance to fluconazole is often associated with drug efflux mechanisms and mutations in the ERG11 gene[8]. The widespread use of azoles in healthcare and agriculture has exacerbated the issue, emphasizing the need for careful antifungal stewardship and regional monitoring of susceptibility trends.

Itraconazole showed moderate efficacy, with a mean MIC of 1.57µg/mL and $MIC_{50/90}$ values of 0.5µg/mL and 4µg/mL. Although itraconazole remains a useful treatment for dermatophytosis, our study suggests emerging resistance, as these MIC values are higher than those reported in some earlier studies.

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Geographic differences and increased exposure to azoles may account for the discrepancies in susceptibility patterns.

Voriconazole, which is not commonly used for dermatophytosis, demonstrated excellent *in vitro* activity, with all isolates showing low MIC values. This result supports the potential of voriconazole as a treatment alternative for resistant cases, as it has been proven effective against *Trichophyton* and *Microsporum* species.

Griseofulvin, despite being one of the oldest antifungals, continues to show good activity, though resistance is rising due to repeated or improper use, especially with steroid-containing creams. In our study, griseofulvin showed comparatively lower MIC values, indicating better susceptibility than earlier reported. However, the increasing resistance against griseofulvin underscores the need for newer antifungal options.

Terbinafine remains a key drug in dermatophytosis treatment, but resistance is increasing. In our study, the geometric mean MIC was 0.22µg/mL, with MIC_{50} and MIC_{90} values of 0.06µg/mL and 8µg/mL, respectively. Other studies have reported varying resistance rates, with resistance linked to mutations in the squalene epoxidase gene (17). This highlights the need for continued surveillance and the development of strategies to combat terbinafine resistance.

Efinaconazole, a newer-generation triazole, showed excellent in vitro activity, with a low geometric mean MIC of 0.06µg/mL. Despite slight variations in MIC values across studies, efinaconazole demonstrates strong efficacy against dermatophytes, including strains resistant to other antifungals. It is emerging as a promising treatment, particularly for cases resistant to first-line agents. Recent studies have shown that treating co-existing fungal infections, such as tinea pedis, can lead to better clinical outcomes in onychomycosis treatment. However, given the potential for resistance, efinaconazole should be used judiciously, with ongoing monitoring of susceptibility patterns[4].

Among the two species isolated, *T. mentagrophytes* complex exhibited higher MICs compared to *T. rubrum*. This finding is consistent with other studies, suggesting that *T. mentagrophytes* complex strains are more likely to show resistance. Early identification of the species is crucial for effective treatment, as *T. rubrum* strains were generally more susceptible to drugs like terbinafine and griseofulvin.

Given the emergence of resistance and recalcitrant dermatophytosis, performing AFST is vital. It helps clinicians choose the most effective antifungal agent for each patient, ensuring better clinical outcomes. Moreover, by identifying resistant strains early, AFST can play a key role in curbing the further rise of antifungal resistance among dermatophytes. Tailored treatment based on AFST results not only improves patient recovery but also helps mitigate the broader public health challenge of increasing resistance, ultimately preserving the effectiveness of current antifungal therapies.

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