

Antifungal Susceptibility and Biofilm Formation in Dermatophytes from Chronic and Recurrent Dermatophytosis: Correlation with Treatment Failure

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Abstract:

Background: In India, dermatophytosis has become a serious public health issue due to an increasing number of cases that develop into chronic or recurrent illnesses that do not respond well to conventional antifungal treatment. Treatment failure is thought to be largely caused by shifting dermatophyte species epidemiology, growing antifungal resistance, and possible contributing variables including biofilm formation. However, there is still a dearth of systematic data, especially in eastern India, linking antifungal susceptibility patterns, biofilm-forming capacity, and clinical outcomes.

Objectives: The objective of this study was to analyze the biofilm-forming capacity and antifungal susceptibility profile of dermatophyte isolates from patients with recurrent and chronic dermatophytosis, as well as their association with treatment failure that has been recorded.

Methods: Between February 2023 and January 2024, a prospective cross-sectional study was carried out at Nalanda Medical College and Hospital in Patna. A total of 200 patients with clinically diagnosed chronic or recurrent dermatophytosis were enrolled. Fungal culture and direct microscopy were applied to clinical specimens. Phenotypic techniques were used to identify dermatophyte isolates, and when necessary, genetic confirmation was obtained. A standardized broth microdilution approach was employed for antifungal susceptibility testing to ascertain the minimum inhibitory concentrations (MICs) for frequently used antifungal drugs. Using a microtiter plate crystal violet assay, biofilm production was evaluated and classified as weak, moderate, or strong. To investigate correlations with treatment failure, clinical and laboratory indicators were examined.

Results: A significant percentage of cases had dermatophytes identified, with species from the *Trichophyton mentagrophytes/interdigitale* complex predominating. Terbinafine and fluconazole often showed high minimum inhibitory concentrations (MICs), while more recent azoles showed relatively lower MICs. A considerable percentage of isolates demonstrated moderate to strong biofilm-forming ability. Strong biofilm producers were strongly linked to previous treatment failure and were more likely to have increased MICs.

Conclusion: The research reveals a worrying pattern of decreased antifungal susceptibility in dermatophytes that cause recurring and chronic dermatophytosis. Antifungal resistance and treatment failure seem to be significantly influenced by biofilm development. Incorporating biofilm evaluation and antifungal susceptibility testing into standard diagnostic procedures may assist direct more successful treatment approaches and enhance patient outcomes.

Keywords: Dermatophytosis, Antifungal susceptibility, Biofilm formation, Chronic dermatophytosis, Treatment failure

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Introduction

One of the most prevalent superficial fungal infections in the world, dermatophytosis is especially prevalent in tropical and subtropical areas where heat, humidity, crowding, and a lack of access to medical care all contribute to its spread. Dermatophytosis was once thought to be a curable ailment in India, but over the past ten years, doctors have noticed a significant increase in cases that are chronic, recurrent, and do not respond well to conventional antifungal therapy. Dermatophytosis was once a common outpatient diagnosis, but this changing clinical pattern has made it a difficult therapeutic issue with substantial effects on patient quality of life and medical resources [1,2].

Recurrent dermatophytosis is described as a relapse that occurs shortly after an apparent clinical cure, whereas chronic dermatophytosis is commonly defined as an infection that lasts longer than six months despite receiving appropriate treatment. Inappropriate or prolonged use of topical corticosteroid-antifungal combinations, incomplete treatment courses, poor adherence, reinfection from untreated contacts, and host-related factors like immunological dysfunction and diabetes mellitus have all been linked to this shift [3,4]. Although these behavioral and clinical aspects are well known, there is mounting evidence that microbiological factors are just as significant in treatment failure.

Recent years have seen a notable shift in the epidemiology of dermatophyte species. A

decrease in *Trichophyton rubrum* and an increase in species from the *Trichophyton mentagrophytes/interdigitale* complex, including strains now known as *Trichophyton indotineae*, have been noted in several studies conducted in various parts of India [5,6]. Because these new species have been linked to more widespread illness, increased recurrence rates, and decreased sensitivity to widely used antifungal medications, especially terbinafine, this change is therapeutically significant.

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Biofilm development has been identified as a potential factor regulating antifungal tolerance and infection persistence, going beyond traditional resistance mechanisms. Biofilms are organized groups of microorganisms enmeshed in an extracellular matrix that can improve survival in harsh environments, change metabolic activity, and obstruct drug

penetration [9]. While biofilm formation in yeasts like *Candida* has been extensively researched, its function in dermatophytes has gotten relatively less attention. The discrepancy between in vitro susceptibility results and clinical outcomes may be partially explained by dermatophyte biofilms, which may lead to decreased antifungal susceptibility, according to preliminary data [10].

Even while antifungal resistance and biofilm formation are becoming more widely recognized, there is still a dearth of information, especially from eastern India, linking these laboratory results to clinical therapy failure in chronic and recurrent dermatophytosis. The majority of research that are currently available either concentrate on epidemiological trends or susceptibility patterns without incorporating biofilm assessment and thorough clinical correlation. The development of evidence-based treatment plans and the prudent use of antifungal medications depend on closing this gap.

In this regard, the current study was conducted at a tertiary care facility in Bihar to assess the biofilm-forming capacity and antifungal susceptibility patterns of dermatophyte isolates from patients with recurrent and chronic dermatophytosis, as well as to investigate their correlation with treatment failure. Such integrated study may assist direct more successful therapeutic therapy and offer insightful information about the microbiological underpinnings of persistent dermatophytosis.

Aims and Objectives

Aim

To assess the biofilm-forming capacity and antifungal susceptibility profile of dermatophyte isolates from patients with recurrent and chronic dermatophytosis, and to associate these results with clinical therapy failure.

Objectives

1. To use established phenotypic techniques to identify dermatophyte species isolated from cases of recurring and chronic dermatophytosis, with molecular confirmation when necessary.
2. To ascertain these isolates' in vitro antifungal susceptibility patterns to both modern and widely used antifungal medications.
3. To evaluate dermatophyte isolates' ability to form biofilms and categorize them as weak, moderate, or strong biofilm makers.
4. To examine the relationship between reported treatment failure, antifungal susceptibility patterns, and biofilm formation.
5. To assess treatment-related and clinical factors that lead to biofilm development and antifungal resistance.

Materials and Methods

Study Design and Setting

This prospective cross-sectional study was carried out at the microbiology and dermatology departments of Nalanda Medical College and Hospital (NMCH), Patna, a tertiary care teaching hospital serving a sizable population from Bihar's rural and urban areas. The investigation was conducted between February 2023 and January 2024.

Study Population and Sample Size

During the trial period, 200 patients with a clinical diagnosis of recurrent or chronic dermatophytosis were included. Recurrent dermatophytosis was defined as a relapse that happened within six weeks of an apparent clinical cure following appropriate therapy, and chronic dermatophytosis was described as an infection that persisted for more than six months despite treatment. The sample size was determined to have sufficient power to identify a statistically significant correlation between treatment

failure, biofilm formation, and antifungal resistance.

Inclusion and Exclusion Criteria

As long as they met the requirements for chronic or recurring disease and provided informed consent, patients of all ages and genders who presented with clinically suspected dermatophytosis involving skin, hair, or nails were included. The study excluded patients with non-fungal dermatoses, those who had received systemic antifungal medication during the previous two weeks, and cases in which sufficient clinical data could not be acquired.

Clinical Data Collection

A standardized proforma was used to record comprehensive clinical data. Demographic information, the length and location of the infection, the size of the lesions, the history of recurrence, previous use of topical corticosteroids and antifungals, treatment compliance, and the existence of related comorbidities such as diabetes mellitus were all included. Persistence or worsening of lesions despite proper antifungal therapy recommended by a trained healthcare provider was considered clinical treatment failure.

Specimen Collection and Direct Microscopy

Clinical specimens, such as hair samples, nail clippings, and skin scrapings, were taken aseptically from the active borders of lesions following 70% alcohol cleaning. Fungal hyphae, arthroconidia, or spores were found by direct microscopic examination using a 10–20% potassium hydroxide (KOH) mount.

Fungal Culture and Identification

Samples were incubated at 25–30°C for a maximum of four weeks after being inoculated onto Sabouraud dextrose agar (SDA) with and without cycloheximide. Periodically, cultures were checked for fungal development. Lactophenol cotton blue mounts were used to identify

dermatophyte isolates based on microscopic features, colony shape, and pigmentation. When necessary, additional tests including the urease production and hair perforation tests were carried out. Internal transcribed spacer (ITS) region sequencing was used for molecular identification of isolates with unclear phenotypic characteristics.

Antifungal Susceptibility Testing

A standardized broth microdilution method was used for antifungal susceptibility testing in compliance with CLSI M38 standards. For both more recent antifungal drugs like luliconazole and posaconazole as well as more widely used ones like terbinafine, itraconazole, fluconazole, and griseofulvin, minimum inhibitory concentrations (MICs) were calculated. After incubation, MIC endpoints were visually read, and reference strains were used to guarantee quality control.

Assessment of Biofilm Formation

A microtiter plate crystal violet assay was used to evaluate the dermatophyte isolates' capacity to create biofilms. In short, 96-well plates were inoculated with standardized fungal solutions and incubated under carefully monitored conditions. Following incubation, wells were cleaned, fixed, and stained with crystal violet before the absorbance was determined using spectrophotometry. Isolates were classified as weak, moderate, or robust biofilm producers based on optical density values.

Quality Control

Standard operating procedures were followed in every laboratory procedure. To ensure reliability, biofilm tests and susceptibility testing were performed twice. Clinical outcome data were blinded to laboratory workers conducting biofilm and susceptibility assessments.

Statistical Analysis

The proper statistical software was used to enter and analyze the data. Demographic, clinical, and laboratory results were

summarized using descriptive statistics. Whereas continuous variables were examined using non-parametric tests when necessary, categorical variables were compared using chi-square or Fisher's exact test. Spearman's correlation coefficient was used to evaluate the relationship between biofilm formation and antifungal MIC values. Statistical significance was defined as a p-value of less than 0.05.

Results

Study Population and Clinical Characteristics

The study comprised 200 patients with chronic or recurrent dermatophytosis who had been clinically diagnosed. The study cohort was predominately male (62%, n = 124), with a mean age of 34.6 ± 12.8 years. 118 cases (59%) had chronic dermatophytosis, while 82 cases (41%) had recurrent infections. Tinea corporis and cruris, either by themselves or in combination, were the most impacted areas. 86% of patients had previously used antifungals, and 64% had used topical steroid-containing combinations. Diabetes mellitus was the most common comorbidity found in 28% of individuals.

Table 1. Demographic and Clinical Profile of Study Participants (n = 200)

Variable	Number (%)
Age (years), mean \pm SD	34.6 \pm 12.8
Male	124 (62)
Female	76 (38)
Chronic dermatophytosis	118 (59)
Recurrent dermatophytosis	82 (41)
Prior antifungal use	172 (86)
Topical steroid misuse	128 (64)
Diabetes mellitus	36 (18)
Multiple site involvement	94 (47)

Mycological Findings

In 164 cases (82%), direct microscopy with KOH mount yielded positive results. In 152 cases (76%), fungal culture produced dermatophytes, which were then subjected to additional laboratory investigation.

Trichophyton rubrum (26.3%) and *Trichophyton mentagrophytes/interdigitale* complex (64.5%) were found to be the most common species. Less frequently isolated species included *Microsporum gypseum* and *Epidermophyton floccosum*.

Table 2. Distribution of Dermatophyte Species Isolated (n = 152)

Species	Number (%)
<i>Trichophyton mentagrophytes/interdigitale</i> complex	98 (64.5)
<i>Trichophyton rubrum</i>	40 (26.3)
<i>Microsporum gypseum</i>	8 (5.3)
<i>Epidermophyton floccosum</i>	6 (3.9)

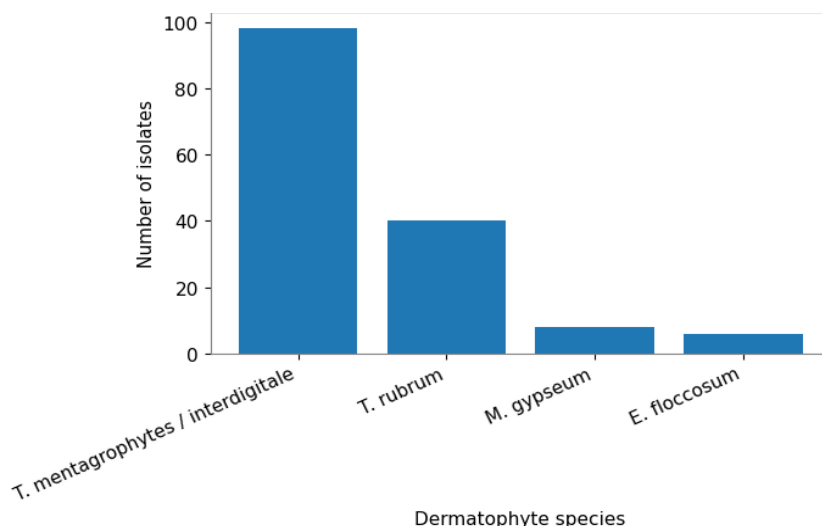


Figure 1: Distribution of dermatophyte species isolated from chronic and recurrent dermatophytosis.

Antifungal Susceptibility Patterns

High minimum inhibitory concentrations (MICs) for widely used antifungals were shown by antifungal susceptibility testing. 69.1% of isolates showed decreased susceptibility to terbinafine, especially T. mentagrophytes/interdigitale complex.

Additionally, a significant percentage of isolates had increased MICs for griseofulvin and fluconazole.

Newer azoles, such luliconazole and posaconazole, on the other hand, showed superior in vitro action with lower MIC values.

Table 3. Antifungal Susceptibility Profile of Dermatophyte Isolates (n = 152)

Antifungal Agent	MIC Range (µg/mL)	Isolates with High MICs n (%)
Terbinafine	0.03 – ≥8	105 (69.1)
Fluconazole	0.5 – ≥64	92 (60.5)
Griseofulvin	0.25 – ≥8	74 (48.7)
Itraconazole	0.03 – 4	46 (30.3)
Luliconazole	0.004 – 0.25	18 (11.8)
Posaconazole	0.016 – 0.5	14 (9.2)

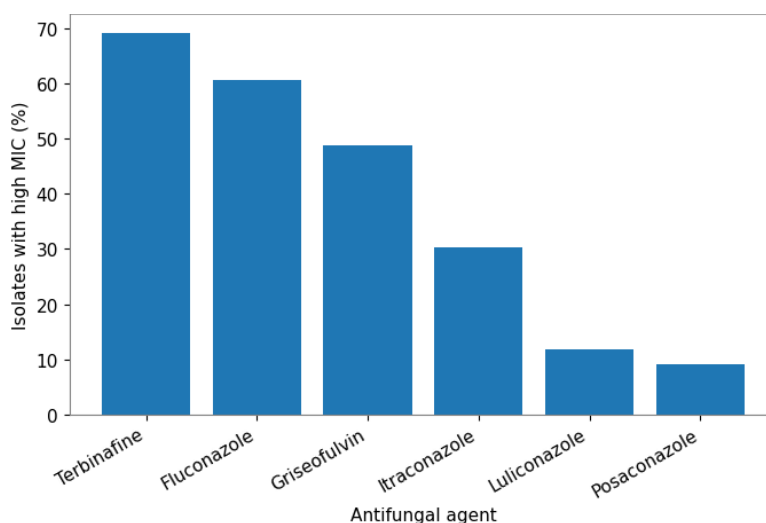


Figure 2: Proportion of dermatophyte isolates showing high minimum inhibitory concentrations to antifungal agents.

Biofilm Formation

A considerable percentage of dermatophyte isolates were shown to produce biofilms. 38.2% of isolates showed strong biofilm production, whilst 34.9% produced

moderate biofilms. 26.9% of isolates showed little or no biofilm development.

Compared to recurrent cases, isolates from individuals with chronic dermatophytosis had a considerably higher frequency of strong biofilm development ($p < 0.05$).

Table 4. Biofilm-Forming Capacity of Dermatophyte Isolates (n = 152)

Biofilm Category	Number (%)
Strong	58 (38.2)
Moderate	53 (34.9)
Weak / None	41 (26.9)

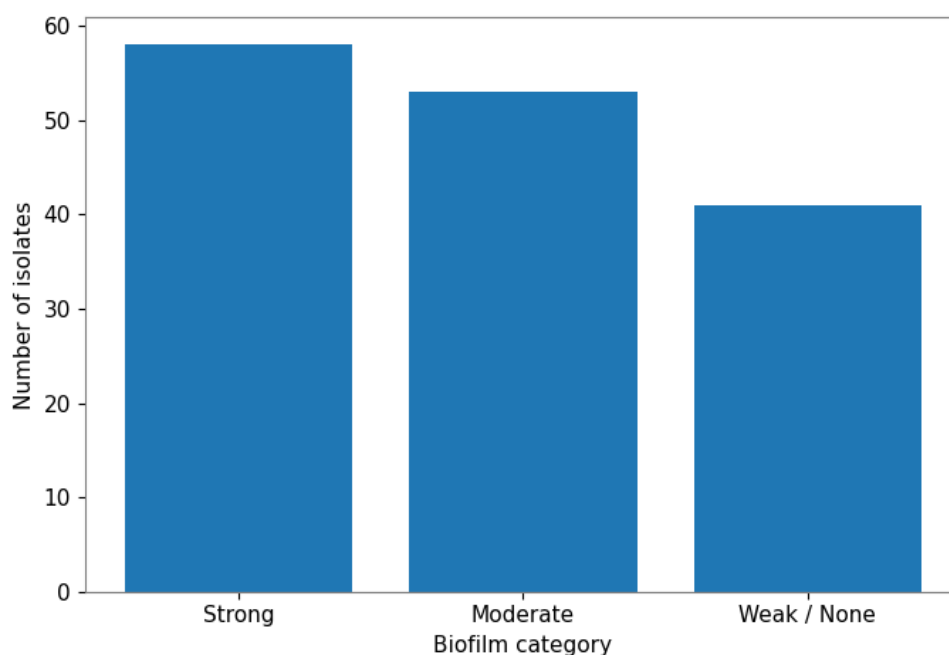


Figure 3: Biofilm-forming capacity of dermatophyte isolates assessed by microtiter plate assay

Correlation Between Biofilm Formation, Antifungal Resistance, and Treatment Failure

Strong biofilm formation was shown to be associated with higher MICs for terbinafine and fluconazole ($p < 0.01$). Strong biofilm-producing isolates were more often linked

to verified clinical treatment failure (72.4%) than moderate (51.9%) and weak biofilm-producing isolates (29.3%). Terbinafine MIC values and biofilm biomass showed a favorable association ($r = 0.61$, $p < 0.001$), according to Spearman correlation analysis.

Table 5. Association of Biofilm Formation with Treatment Failure (n = 152)

Biofilm Category	Treatment Failure n (%)
Strong	42 (72.4)
Moderate	28 (51.9)
Weak / None	12 (29.3)

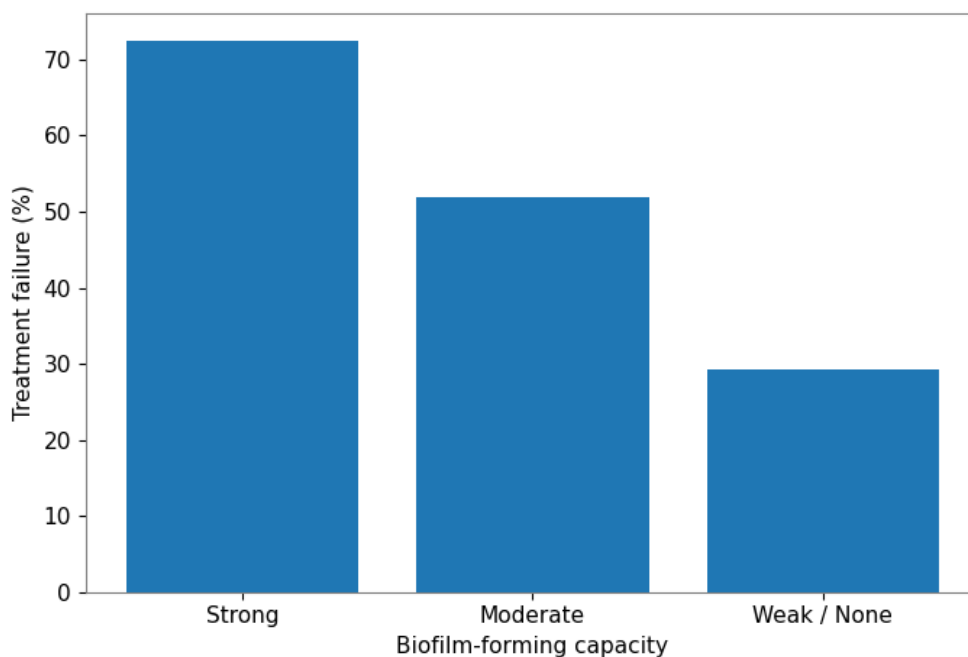


Figure 4: Relationship between biofilm-forming capacity and clinical treatment failure

Discussion

The present study provides a comprehensive evaluation of antifungal susceptibility patterns and biofilm-forming ability of dermatophytes isolated from patients with chronic and recurrent dermatophytosis and correlates these laboratory findings with clinical treatment failure. The results highlight a multifactorial basis for persistent dermatophytic infections, where microbiological factors appear to play a central role alongside clinical and behavioral contributors.

The prevalence of species from the *Trichophyton mentagrophytes/interdigitale* complex, which made up about two-thirds of all isolates, was a noteworthy finding of this investigation. This finding is consistent with recent observations from various parts of India that show a move away from *Trichophyton rubrum* and toward the *T. mentagrophytes/interdigitale* complex, especially in persistent and resistant infections [11,12]. Because these newly emerging species are increasingly linked to aggressive disease, widespread involvement, and decreased responsiveness to conventional antifungal treatments, this

epidemiological shift is therapeutically significant.

More than two-thirds of isolates showed higher minimum inhibitory concentrations (MICs) for terbinafine, according to the antifungal susceptibility results. Similar patterns have been shown in multicentric Indian research, where mutations in the squalene epoxidase gene have been connected to terbinafine resistance and decreased medication efficacy [13,14]. The selection pressure causing resistance may have been exacerbated by the widespread use of terbinafine as a first-line medication, frequently at inadequate doses or durations. The study's high minimum inhibitory concentrations (MICs) for fluconazole and griseofulvin highlight the medicines' limited use in chronic or recurrent infections, as other researchers have previously reported [15].

Newer azoles, such as luliconazole and posaconazole, on the other hand, showed lower MIC values against the majority of isolates, indicating better in vitro action. These results are in line with previous research that shown that newer azoles had strong action against dermatophytes, including strains that are less susceptible to

traditional treatments [16,17]. However, cost, availability, and long-term safety must be carefully taken into account for these agents to be clinically applicable, especially in settings with limited resources.

The evaluation of biofilm formation and its relationship to antifungal resistance and treatment failure is a significant strength of the current investigation. Strong biofilm production was strongly linked to higher MICs and clinical non-response, and a significant percentage of isolates produced moderate to strong biofilms. Even in the absence of traditional genetic resistance pathways, biofilms are known to function as a physical and metabolic barrier, decreasing antifungal penetration and fostering phenotypic tolerance [18]. Although biofilm development in *Candida* species has been thoroughly investigated, evidence on dermatophyte biofilms is beginning to surface, and the present findings contribute to the expanding corpus of research demonstrating their clinical significance [19].

According to the positive link seen between terbinafine MIC values and biofilm biomass, biofilm formation may increase antifungal tolerance, which could lead to chronic infection even with seemingly effective treatment. Biofilm-forming dermatophytes showed decreased susceptibility to several antifungal drugs in experimental and clinical investigations [20]. The frequent discrepancy between in vitro susceptibility results and clinical outcomes seen in ordinary therapy may be partially explained by this.

Patients with treatment failure in this study frequently had clinical variables like comorbidities, abuse of topical corticosteroid combinations, and prior exposure to antifungals. These results are consistent with previous studies that highlight how unreasonable steroid use and unfinished treatment regimens contribute to the persistence of chronic dermatophytosis [21, 22]. In addition to suppressing local immune responses, steroids may change the

dynamics of fungal development, which could promote the creation and persistence of biofilms.

When considered collectively, the results of this study indicate that treatment failure in chronic and recurrent dermatophytosis is substantially impacted by changing dermatophyte biology, including antifungal resistance and biofilm formation, rather than being exclusively due to patient-related factors. Therefore, routine species identification, testing for antifungal susceptibility, and taking biofilm-associated tolerance into account may be crucial for optimizing treatment approaches in instances that are resistant to treatment. Future research utilizing in vivo biofilm models and molecular study of resistance pathways may improve knowledge and direct tailored antifungal treatment.

Conclusion

The current investigation shows that among dermatophyte isolates, considerable antifungal resistance and increased biofilm-forming capacity are progressively linked to chronic and recurring dermatophytosis. High minimum inhibitory concentrations to widely used antifungal drugs, especially terbinafine and fluconazole, were found together with a predominance of organisms from the Trichophyton mentagrophytes/interdigitale complex. The increasing frequency of treatment failure observed in standard clinical practice is explained by these facts.

One significant microbiological aspect linked to antifungal tolerance and infection persistence is biofilm development. Strong biofilm-forming isolates were substantially linked to higher MIC values and greater rates of clinical non-response, indicating that biofilms may be important in decreasing treatment efficacy even in the absence of obvious genetic resistance. The study emphasizes the necessity of managing recurring and chronic dermatophytosis in a more logical and evidence-based manner. Treatment results

may be enhanced by including antifungal susceptibility testing, being aware of newly emerging resistant species, and using antifungal medicines sparingly. It is equally important to address contributing factors such as poor treatment adherence and topical corticosteroid usage. To improve treatment recommendations and reduce the growing prevalence of refractory dermatophytosis, further multicentric research incorporating molecular resistance pathways and *in vivo* biofilm models is necessary.

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