Is Antifungal Resistance a Cause for Treatment Failure in Dermatophytosis: A Study Focused on Tinea Corporis and Cruris from a Tertiary Centre?

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Abstract

Background: Dermatophytoses are one of the most common skin diseases that have been largely simple to treat. However, in recent years, these infections have become recalcitrant to treatment which can possibly be due to antifungal resistance. **Aim:** To analyze the resistance pattern of patients with recalcitrant dermatophytoses. **Materials and Methods:** A cross-sectional evaluation was undertaken of 40 consecutive patients with recalcitrant tinea corporis/cruris/both who had taken systemic antifungal treatment and did not respond completely to therapy or had recurrent lesion within 1 month of stopping the therapy. Terbinafine, fluconazole, itraconazole, ketoconazole, amphotericin B, and voriconazole were the antifungals tested using broth microdilution assay for antifungal susceptibility testing of dermatophytes, and MIC50, 90 values were recorded. **Results:** KOH mount was positive in 18 (45%) patients, culture was positive in 28 (70%) patients. *Trichophyton mentagrophytes* (35%) and *T. rubrum* (27.5%) were the predominant isolates. Overall, activity of terbinafine and itraconazole were significantly higher than the other drugs tested. For terbinafine, both *T. mentagrophytes* and *T. rubrum* were inhibited at MIC_{90} of 0.125 µg/ml. Itraconazole-inhibited *T. mentagrophytes* and *T. rubrum* at MIC_{90} of 0.0625 and 0.25 µg/ml, respectively. All isolates had reduced susceptibility to fluconazole. **Conclusion:** While MIC seen were higher than western data, *in-vitro* resistance (>1 µg/ml) to antifungals was not seen and probably may not be a cause of treatment failure. Possibly, treatment failure lies in the intricate host fungal interaction and virulence of species which help it to evade host immune response.

Keywords: *Antifungals, dermatophytosis, itraconazole, MIC, resistance, tinea, terbinafine*

Introduction

Dermatophytes are pathogenic fungi that have the capacity to invade keratinized structures such as skin, hair, and nails. These infections are known as dermatophytoses and are caused by species of three genera – *Trichophyton*, *Epidermophyton*, and *Microsporum*. Based on their natural habitat, they are classified into three groups – geophilic, zoophilic, and anthrophilic species.

Dermatophytosis is one of the most common skin diseases worldwide, especially in tropical countries like India. Various antifungal agents have been used for the treatment of these infections. Most common systemic agents used are terbinafine, fluconazole, and itraconazole. Although there is a rising trend of patients who tend to relapse following cessation of antifungal therapy, the relapses have not been conclusively proven to be consequent to resistance. Here, it is important to appreciate that routine antifungal susceptibility testing is not being carried out in dermatophyte infections in India. Possibly, there could be other factors that play a role including the host immune response and the barrier function of the epidermis. The use of corticosteroids in fixed dose combination (FDC) with antifungals and their misuse may also account for such relapses.

The aim of this study was to analyze the resistance pattern of patients with recalcitrant dermatophytosis.

Materials and Methods

This study was carried out in a tertiary hospital in Delhi. A cross-sectional evaluation was undertaken of 40 consecutive patients aged more than 18 years, who presented with lesions suggestive of Tinea corporis/cruris/both,

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who had taken antifungal treatment, topical and/or systemic including one complete course of systemic fungicidal treatment (terbinafine 250 mg once daily/itraconazole 100 mg twice daily for 4 weeks), and did not either respond completely to therapy (judged on the basis of clinical response) or had a recurrent lesion within 1 month of stopping the therapy. The latter duration was based on studies that show that terbinafine and itraconazole levels persist in the epidermis for 3 weeks after therapy.[1,2] We excluded patients with tinea involving other body sites except those mentioned above. The study was approved by the Institutional Review Board, and is in continuation of a previous study.[3]

Specimen collection

The skin was disinfected with alcohol. With the help of a sterile scalpel blade, the skin was scraped from the center to the edge. The samples were collected in a black paper and direct examination was done using potassium hydroxide (KOH) mount and cultured in Sabouraud's dextrose agar with dermatophyte test medium and incubated at 37°C and 25°C for up to 6 weeks.

The skin scrapings were kept in 10% KOH for 15–20 minutes and examined under the microscope. The fungus was seen as branching hyaline mycelia. Culture media were checked for growth every 3 days, and fungal growth was identified by slide culture techniques, as per the standard diagnostic procedures.

Antifungal drugs

The following antifungal agents were used in this study: terbinafine, fluconazole, itraconazole, ketoconazole, amphotericin B, voriconazole (Pfizer Inc., New York, N.Y.), fungisome (1 mg Amp intercalated in Liposomes Life care innovation Pvt Ltd Gurgaon). The drugs were obtained as reagent grade powders or in liquid form and preserved according to the manufactures' instructions. Dimethyl sulfoxide (DMSO) was used for the first dilution of antifungal powders.

Antifungal susceptibility testing

(i) Microdilution method: The broth microdilution assay for antifungal susceptibility testing of dermatophytes was previously developed as a modification of the National Committee for Clinical Laboratory Standards (NCCLS) M38-A2 method.[4] RPMI 1640 medium (Himedia) with L-glutamine but without sodium bicarbonate and buffered at pH 7.0 with 3-(*N*-morpholino) propanesulfonicacid (Himedia), monosodium salt, was the medium used for broth microdilution susceptibility testing. Serial twofold dilutions were prepared by the NCCLS M38-A2 method. The final concentration of fluconazole was 0.125–64 μg/ml, whereas those of itraconazole, terbinafine, voriconazole, ketoconazole, fungisome, were 0.015–16 μg/ml. Based on a study with inter-laboratory comparison and validation, a minimum inhibitory concentration(MIC) range for standard dermatophytic strains (taken from the Ghannoum laboratory) was determined [Table 1] and used to compare our data.[5] Usual MICs are 0.03 μg/ml in susceptible strains of *T. rubrum*; in these resistant strains, MICs were >1.0 μg/ml.[6]

A standardized inoculum was prepared by counting the microconidia microscopically. Cultures were grown on Potato Dextrose Agar slants for 4 days at 35°C to produce conidia. Sterile normal saline (85%) was added to the agar slant, and the cultures were gently swabbed with a cotton-tipped applicator to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile tube, and the volume was adjusted to 5 ml with sterile normal saline. The resulting suspension was counted on a hemocytometer and diluted in RPMI 1640 medium to the desired concentration. Microdilution plates were set up in accordance with the NCCLS M38-A reference method. Column 12 was filled with 200 μl of medium to serve as a sterility control. Columns 1 through 10 were filled with 100 μl of the inoculum and 100 μl of the serially diluted antifungal agent. Column 11 was filled with 100 μl of the inoculum and 100 μl of media served as a growth control. The microdilution plates were incubated at 35°C and were read visually after 4 days of incubation. The MIC was defined as the concentration at which the growth of the organism was inhibited up to 80% compared with the growth in the control well. All isolates were run in duplicate, and the results were read visually.

In addition, MIC values at which 50% and 90% of isolates were inhibited (MIC_{50} and MIC_{90}, respectively) were recorded. All isolates when cultivated in the absence of antifungals produced clearly detectable growth after 5 days of incubation.

Analysis

The species were identified and MIC_{90} was determined and compared with the previous studies and standards. The difference in the MIC_{90} values between the species identified and the antifungal agents was compared using *t*-test; $P < 0.05$ was significant.

Table 1: Reference MIC range of antifungals used in the study

Dermatophyte QC	Antifungal agent	MIC range (μg/ml)
T. mentagrophytes	Ciclopirox	0.5-2.0
ATCC MYA-4439	Griseofulvin	0.12-0.5
	Itraconazole	0.03-0.25
	Posaconazole	0.03-0.25
	Terbinafine	0.002-0.008
	Voriconazole	0.03-0.25
T. rubrum	Ciclopirox	0.5-2.0
ATCC MYA-4438	Fluconazole	0.5-4.0
	Voriconazole	0.008-0.06

Results

The study included 40 patients in the age group of 18–55 years. Of these, 23 were males and 27 were females. The average age of presentation was 33.6 years. The average duration of disease was 4.5 months. Twenty patients (50%) had tinea corporis, 6 (15%) had tinea cruris, whereas 14 (35%) presented with both. All patients reported history of use of antihistamines and topical steroids, some of these were documented by visual identification and in others was confirmed based on the brand names reported by the patient. The mean duration of treatment with topical steroids was 3.2 months. Twenty-four (60%) patients had family history of tinea infection whereas atopy was present in 4 (10%) patients. Five patients (12.5%) had history of diabetes and were on treatment for the same.

KOH mount was positive in 18 (45%) patients whereas culture was positive in 28 (70%) patients. *T. mentagrophytes* was the most common species and was isolated in 35% of the patients whereas *T. rubrum* was found in 27.5% of the cases.

Overall, the activity of terbinafine and itraconazole were significantly higher than the other drugs tested [Table 2]. For terbinafine, both *T. mentagrophytes* and *T. rubrum* were inhibited at MIC_{90} of 0.125 μg/ml. Itraconazole inhibited *T. mentagrophytes* and *T. rubrum* at MIC_{90} of 0.0625 and 0.25 μg/ml, respectively. All isolates had reduced susceptibility to fluconazole, which was demonstrated by the fact that MIC values for this drug were higher than the other agents. Amphotericin B had an MIC comparable to that of terbinafine for *T. rubrum* but was higher for *T. mentagropohytes*. The MIC_{90} values did not reveal any marked difference in the antifungals tested between *T. mentagrophytes* and *T. rubrum* except for itraconazole and fluconazole. Using the existing standards and the accepted cut-off of >1 μg/ml for terbinafine, no case of resistance to terbinafine was detected.

There was a statistically significant difference between the MIC of terbinafine and fluconazole, and terbinafine and voriconazole for *T. rubrum* ($P > 0.005$). In case of *T. mentagrophytes*, a similar difference in MIC was noted between MIC of terbinafine and fluconazole, itraconazole, and voriconazole.[2]

On comparing the MIC_{90}, the order of potency of antifungal drugs was voriconazole>terbinafine/amphotericin B/ketoconazole>itraconazole>fluconazole for *T. rubrum*. In case of *T. mentagrophytes*, the order of potency was voriconazole/itraconazole>>terbinafine/ketoconazole>amphotericin B>fluconazole.

Discussion

Our results [Table 2] reveal that there was no resistance to systemic terbinafine and itraconazole using a cut off value of 1 μg/ml, though the high MIC to fluconazole suggests that dermatophytes are possibly resistant to this drug. Further, amphotericin B was found to have a similar MIC_{90} to terbinafine, and this may have a potential use as a topical agent in dermatophyte infections.[7] Though voriconazole had the lowest MIC, its serum levels vary widely among patients due to differences in the metabolism, posing risk of toxicity or therapeutic failure. Moreover, it is pertinent to point out that this drug has very poor skin levels and may not have *in-vivo* utility. We have largely restricted our discussion on *T. mentagrophtes* and *T. rubrum*, though a few cases of other species were also seen [Table 2]. An analysis of salient studies with the MIC of various antifungal drugs against *T. rubrum* and *T. mentagrophytes* [Table 3][5,8-13] were used to compare our results.

In case of terbinafine, our MIC_{90} values are higher than previous studies [Table 3]; however, it is similar to the study from Turkey. Notably, the MIC of itraconazole largely adheres to the MIC values from most published studies [Table 3]. Notably, the trend shows that the order of efficacy of oral antifungals from the highest to lowest MIC is terbinafine > itraconazole > fluconazole [Table 3]. Though *T. rubrum* follows this trend, in case of *T. mentagrophytes,* itraconazole was found to have a lower MIC. Interestingly, no case of terbinafine resistance was seen using the cut-off value of 1 μg/ml, though it is possible that further studies from India might arrive at a different cut-off MIC. For now, our data conforms to the existing data [Table 3] and disregards this as a cause of treatment failure.

T. rubrum is the most common cause of dermatophytosis and onychomycosis in developed countries while in India, the most common emerging species is *T. mentagrophytes*[3] In our study, the latter was more common. This contrasts with the finding of previous authors who have shown that *T. rubrum* was the most common cause.[3,14-16] Direct KOH mount was positive in only 45% of the patients. This is

Table 2: *In vitro* susceptibility (indicated by MIC90) with 6 antifungal drugs against two species of dermatophytes isolated

Organism*	Terbinafine	Fluconazole	Amphotericin B	Itraconazole	Ketaconazole	Voriconazole
	MIC90	MIC90	MIC90	MIC90	MIC90	MIC90
T. mentrogrophyte (14)	0.125	4	0.625	0.0625	0.125	0.0625
	Terbinafine vs Fluconazole and Terbinafine vs Itraconazole and Terbinafine vs Voriconazole (P<0.05)					
T. rubrum (11)	0.125	8	0.125	0.25	0.125	0.0125
	Terbinafine vs Fluconazole and Terbinafine vs Voriconazole (P<0.05)					

*Values are in μg/ml

Table 3: A summary of salient studies showing the MIC of various antifungal drugs against *T. rubrum* and *T. mentagrophytes*

Study	Region	Species	MIC 50 (µg/ml)				
			Terbinafine	Itraconazole	Ketoconazole	Fluconazole	Voriconazole
Adimi *et al*.[8]	Iran	*T. rubrum* (*n*=89)	0.031	0.062	1	32	0.12
		T. mentagrophytes (*n*=136)	0.031	0.06	2	64	0.5
Fernandez-Torres *et al*.[9]	UK	*T. rubrum* (*n*=144)	0.01	0.125	0.12	4	0.06
		T. mentagrophytes (*n*=122)	0.06	0.25	0.5	16	0.25
Gupta *et al*.[10]	Canada	*T. rubrum* (*n*=68)	0.003	0.06	0.06	-	-
		T. mentagrophytes (*n*=14)	0.003	0.06	0.25	-	-
		T. tonsurans (*n*=5)	0.003	0.06	0.25	-	-
Ghannoum *et al*.[5]		*T. rubrum*	0.03	0.125		16	0.06
Jessup *et al*.[11]	Ohio	*T. rubrum* (*n*=152)	0.001	0.13	-	1	-
		T. mentagrophytes (*n*=32)	0.001	<0.06	-	2	-
		T. tonsurans (*n*=42)	1	<0.06	-	1	-
Yenisehirli[12]	Turkey	*T. rubrum*	0.125	0.5	0.5		
		T. mentagrophytes	0.125	0.5	1		
da Silva Barros[13]	Brazil	*T. rubrum*	0.007	0.25		32	
		T. mentagrophytes	0.015	0.125		64	
Present study	India	*T. rubrum*	0.125	0.25	0.125	8	0.0125
		T. mentagrophytes	0.125	0.0625	0.125	4	0.0625

more than a previous study where 35.7% of cases were positive,[3] conforming to the accepted range of 23.8% to as high as 91.2%.[17] We incubated the culture both at 37°C and 25°C and achieved an isolation rate of 70%, which is better than a previous study (39%).[3] Various studies have used a temperature of 28°C and achieved better growth.[13,18]

Superficial fungal infections had always been simple to treat with the basket of antifungal agents available; however, recently there has been a sudden increase in the number of patients with recalcitrant infection. Recalcitrant dermatomycosis refers to relapse, recurrences, reinfection, persistence, and possibly microbiological resistance.[19] It is mainly seen with truncal and crural tinea infections. Hence, we confined our study to this group of patients.

Resistance is a microbiological term and is used when the MIC of the fungal species isolated is more than >1 µg/ml for terbinafine. As the different antifungal agents have varying efficacy against various dermatophytes, it is pertinent to carry out antifungal susceptibility testing before labelling "drug resistance" as the cause of failure of therapy. Such studies will help the clinician in managing the disease in a better and more evidence based manner. Resistance is a possibility when a case presents with persistent infection or relapses within 4 weeks of an adequate dose regimen of an antifungal drug. This is based on the skin phamacokinetics (pk) of the major drugs used in dermatophytosis. After administration of terbinafine 250 mg daily for 12 days, drug concentrations above the MIC for most dermatophytes may be present for 2–3 weeks after oral therapy is discontinued.[1] Itraconazole may persist in the stratum corneum for 3–4 weeks after discontinuation of therapy.[20] In an *ex-vivo* model, the therapeutic effect of itraconazole in the stratum corneum remained for 2–3 weeks after stopping therapy.[21] Thus, an infection that recurs within 4 weeks after adequate oral therapy can possibly be due to resistance, though we cannot discount the additional role of immune compromise.

Though our work shows a high MIC to terbinafine and itraconazole compared to other studies, no case of microbiological resistance was seen [Table 2]. The former could represent the widespread and sometimes unapproved higher doses administered by clinicians for these disorders and could represent prevalent prescription practices.

Our work concluded that *in-vitro* resistance to antifungals is not common, and thus such cases should not be labelled as "resistant" cases, and the better term to use at present is "recalcitrant infection."

Moreover, *in-vitro* analysis of data is fraught with major lacunae in terms of clinical applicability of results.[22] The information provided by standard antifungal susceptibility test (AFST) methods, MIC, or the disk zone diameter, may not always have clinical relevance in the care of patients with fungal infection. It is this issue (the "clinical utility" or "clinical relevance" of AFST) that is rarely discussed. An important paper had articulated several important principles to consider when discussing the clinical utility of susceptibility test methods. These principles include an understanding that the MIC is a construct that is largely defined by testing conditions rather than a physical or chemical measurement. This measure might correlate with clinical outcome, but a multitude of factors related to the host (immune response, underlying illness, site of infection), the infecting organism (virulence), and the antifungal agent (dose, pharmacokinetics, pharmacodynamics, skin

levels, drug interactions) may be more important than susceptibility test results in determining clinical outcomes for infected patients.[23] Thus, *in-vitro* susceptibility of dermatophytes to antifungal agents does not consistently predict a successful therapeutic outcome.

Thus, there are several other factors which need to be excluded before concluding that a particular antifungal has failed to cure the infection. Infections caused by anthropophilic dermatophytes are generally chronic and accompanied by minimal to varying inflammation.[24] On the other hand, infections with zoophilic fungal species such as *A. benhamiae*, a teleomorph of *T. mentagrophytes*, can induce more severe inflammation in the human host. Thus, it is established that the cutaneous acquired immune responses to dermatophyte infections involve Th1, Th2, and Th17 components, which is in line with other studies involving *Trichophyton spp*.[25] Importantly, a Th2 response leads to persistence of infection. The multiple, functionally distinct signalling pathways in antigen-presenting cells ultimately affect the local Th cell/Treg cell balance, and are likely to be exploited by fungi to allow commensalism or opportunism.

Of the myriad causes listed in Table 4, the most relevant in our study might be the role of steroids that subvert the Th1/Th2 response, the former of which is profoundly inhibited by *T. rubrum*, a major species in our work.[26] The use of topical corticosteroids also affect the antigen receptor recognition capacity of Langerhans' cells and superpotent corticosteroids cause loss of cells expressing Langerhans' cell markers. Moreover, there is a profound effect on immune cytokine production with a reduced production of interleukin-1 (both IL-1α and IL-1β), interferon-γ, tumor necrosis factor alpha, IL-2, and granulocyte–monocyte colony-stimulating factor.[27] Thus the immune suppression caused by *T. rubrum* may be aggravated by the known immunosuppression of steroids.[28,29]

In conclusion, it is important for the clinician to look beyond the antifungal drug, which is small part of the complex immune recognition and interactions determining the ultimate clinical picture of recalcitrant infection.[30]

Table 4: Various factors implicated in recalcitrant dermatophytosis

	Factors
1	Host-fungal immune response (Th1/17 being protective and Th2 causing chronicity of infection)
2	Virulence potential of the infecting strain or species
3	Clinical type of dermatophytosis
4	Barrier defect
5	Local factors: heat, humidity, sweating, type of clothing
6	Pharmacological factors: quality of the drug, compliance, pharmacokinetics and absorption of the drug
7	Reinfection from other sources

Limitations

- We limited our study to tinea corporis and cruris as these are the sites that are recalcitrant to therapy
- Griseofulvin was not included in our battery of antifungals tested as its pharmacokinetics and excretion in the skin are not favorable for dermatophyte infection although few studies have reported low MIC.

Conclusion

In-vitro resistance to antifungals is not common and should not be frequently labelled as a cause of treatment failure. The problem lies in the intricate host fungal interaction and this should be the focus for research.

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Conflicts of interest

There are no conflicts of interest.

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