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Original Research Article

Antifungal susceptibility pattern of dermatomycosis in a tertiary care hospital of North India

Ravika K. Budhiraja[1], Saurabh Sharma[1]*, Sarbjeet Sharma[2], Jasleen Kaur[1], Roopam Bassi[3]

[1]Department of Dermatology, Venereology & Leprosy, [2]Department of Microbiology, [3]Department of Physiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India

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***Correspondence:**
Dr. Saurabh Sharma,
E-mail: drsaurabhsharma@yahoo.co.in

ABSTRACT

Background: Dermatomycoses affect the outer layers of the skin, nails and hair without tissue invasion and are often caused by dermatophytic molds, candida & non dermatophytic molds. Although not dangerous, they are important as a public health problem particularly in the immunocompromised. There are limited studies on the efficacy of antifungal agents against dermatophytes in North India.
Methods: This study was conducted to test the efficacy of 5 systemic antifungal agents viz. voriconazole, itraconazole, terbinafine, fluconazole & griseofulvin using Microbroth dilution technique.
Results: Three different species of dermatophytes which were isolated from the clinically suspected cases were *Trichophyton mentagrophytes*, *T. rubrum* and *M. gypseum*. According to the obtained results, Itraconazole and Voriconazole showed the lowest MIC range while Fluconazole and Griseofulvin had the highest MIC range for most fungi tested.
Conclusions: Despite several treatment options being available for cutaneous fungal infections, due to an inappropriate response, there is an increasing need for determining an antifungal susceptibility profile for specific fungal strains. This will enable the clinician to select an appropriate antifungal agent with minimal side effects to avoid antifungal resistance and treatment failure.

Keywords: Dermatomycosis, Antifungal resistance, Microbroth dilution

INTRODUCTION

Dermatomycoses or superficial fungal infections have shown a monumental increase both in incidence and prevalence in the recent past. Typically, these infections affect the outer layers of the skin, nails and hair without tissue invasion and are often caused by dermatophytic molds belonging to genera *Trichophyton*, *Microsporum* and *Epidermophyton* and sometimes by *Pityriasis versicolor*, candida and non dermatomycotic molds.[1,2] Dermatophytes have not only adapted themselves to animal and human parasitism through evolution but have also developed host specificity ascribed to difference in the composition of keratin.[3] Based on their host specificity dermatophytes are classified into three ecological groups namely *geophiles* (soil), *anthropophiles* (man) and *zoophiles* (animals).

For a long time, the sole anti-dermatophytic agent approved for systemic treatment was griseofulvin.[4] Lately however, it has fallen out of favour due to rise in Griseofulvin-resistant isolates of dermatophytes and existence of strains with elevated MIC levels to Griseofulvin.[5-8] Consequently, allylamines and triazoles

with higher efficacies, less side effects and a shorter duration of treatment have become the mainstay of management of most superficial mycoses.

The emergence of innumerable antifungal-resistant strains and their widespread distribution are the result of many years of underuse, overuse, and misuse of antifungal medication (both topical and oral) and a weak or non-existent antifungal policy and poor infection control.[9] Taking medications by consulting internet instead of doctors, self medication, prescription by quacks and non-dermatologists, non-completion of treatment, easy availability of over the counter drugs and excessive use of anti-fungal pesticides on crops are the main reasons for antifungal resistance.

For in vitro detection of resistance to antifungal agents, there are guidelines given by CLSI (Clinical and Laboratory Standard Institute) document M-38A for broth microdilution method for filamentous fungi, in which MIC (Minimum Inhibitory Concentration) is calculated. Depending on that, a particular drug with a higher MIC is considered relatively resistant.[10]

The studies related to antifungal susceptibility patterns are extremely scarce. Furthermore, the development of an elaborate antifungal profile might contribute to a decreased transmission and impact of resistant fungal strains in the near future.[11] Effective treatment depends on various factors including duration of treatment, appropriate dosage and frequency of application.[12]

A research might contribute in controlling antifungal resistance. Therefore a study to determine the antifungal susceptibility profile of 5 antifungal agents including Fluconazole, Itraconazole, Voriconazole, Terbinafine, and Griseofulvin as per CLSI protocol M38-A, against dermatophytes using Microbroth dilution technique, was conducted in the Department of Dermatology of a tertiary care center of North India.

METHODS

A prospective study was conducted in the Department of Dermatology at Sri Guru Ram Das Medical College, Amritsar from December 2015 to November 2017. A total of 240 patients were included. An approval from the institutional ethics committee was taken. Data was collected in a predesigned format. For patients with sufficient scales, specimen collection, processing, microscopy and culture were done and antifungal susceptibility testing was carried out as per CLSI guidelines by the microbroth dilution technique.

Micro-broth dilution test

In the study, we used double-strength Müeller-Hinton broth (MHB), 4X strength antibiotic solutions prepared as serial two-fold dilutions and the test organism at a concentration of 2×10^6/ml. In a 96 well plate, 100 µl of double-strength MHB, 50 µl each of the antibiotic dilutions and the organism suspension were mixed and incubated at 35°C for 18-24 hours. The lowest concentration showing inhibition of growth was considered the MIC of the organism.

Reading of result

MIC was expressed as the highest dilution which inhibited growth judged by lack of turbidity in the tube. Standard strain of known MIC, run with the test was used as the control to check the reagents and conditions.

Data analysis

Geometric mean (GM), MIC range, MIC50 and MIC90 were obtained for all the isolates tested. MIC50 and MIC90 being the lowest drug concentration, showing 50% and 90% inhibition of growth, respectively. MIC value of antifungal drugs for different species were compared by one-way ANOVA using SPSS version 16 software and a p value <0.05 was considered statistically significant.

RESULTS

A total of 240 clinically suspected cases of superficial fungal infections were selected for microbiological diagnosis. The total number of positive cultures was 59. Final strain identification revealed 41(69.49%) dermatophytes. As shown in Figure 1, among the dermatophytes, *Trichophyton* genus represented 97.6% of the isolates, with *T. mentagrophytes* being the commonest that is 25 (60.98%), followed by *T. rubrum* being 15 (36.58%) and *Microsporum gypseum* being 1 (2.44%).

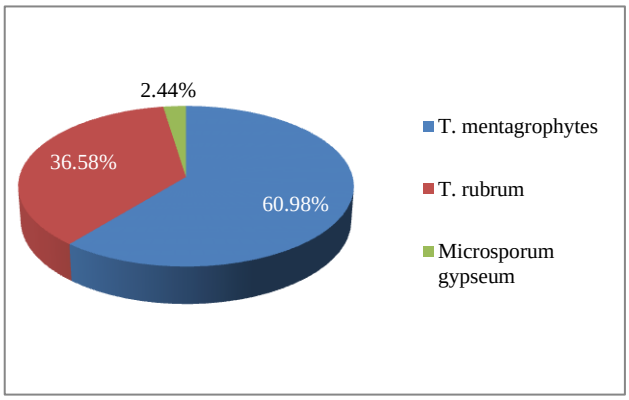

Figure 1: Species wise distribution of dermatophytes.

The MIC distribution, MIC50, MIC90, geometric mean (GM) of Fluconazole for *T. mentagrophytes*, *T. rubrum* and *M. gypseum* is as shown in Table 1.

The MIC distribution, MIC50, MIC90, geometric mean (GM) of Griseofulvin for *T. mentagrophytes*, *T. rubrum* and *M. gypseum* is as shown in Table 2.

Table 1: In vitro susceptibility of dermatophytes to Fluconazole.

	Concentration of Fluconazole (µg/ml)		
	***T. mentagrophytes* (n=25)**	***T. rubrum* (n=15)**	***M. gypseum* (n=1)**
GM	52.48	51.20	64
MIC50	64	64	64
MIC90	128	128	128
Range	32 – 64	32 – 64	NA

*GM=Geometric Mean

Table 2: In vitro susceptibility of dermatophytes to Griseofulvin.

	Concentration of Griseofulvin (µg/ml)		
	***T. mentagrophytes* (n=25)**	***T. rubrum* (n=15)**	***M. gypseum* (n=1)**
GM	1.33	4.67	0.06
MIC50	0.5	4	0.06
MIC90	1	8	0.125
Range	0.03 – 16	2 – 8	NA

*GM=Geometric Mean

Table 3: In vitro susceptibility of dermatophytes to Terbinafine.

	Concentration of Terbinafine (µg/ml)		
	***T. mentagrophytes* (n=25)**	***T. rubrum* (n=15)**	***M. gypseum* (n=1)**
GM	1.33	3.41	0.03
MIC50	0.5	0.5	0.03
MIC90	1	1	0.06
Range	0.03 – 16	0.03 – 8	NA

*GM=Geometric Mean

Table 4: In vitro susceptibility of dermatophytes to Itraconazole.

	Concentration of Itraconazole (µg/ml)		
	***T. mentagrophytes* (n=25)**	***T. rubrum* (n=15)**	***M. gypseum* (n=1)**
GM	0.05	0.06	0.03
MIC50	0.03	0.03	0.03
MIC90	0.06	0.06	0.06
Range	0.03 – 0.125	0.03 – 0.125	NA

*GM=Geometric Mean

Table 5: In vitro susceptibility of dermatophytes to Voriconazole.

	Concentration of Voriconazole (µg/ml)		
	***T. mentagrophytes* (n=25)**	***T. rubrum* (n=15)**	***M. gypseum* (n=1)**
GM	0.04	0.04	0.03
MIC50	0.03	0.03	0.03
MIC90	0.06	0.06	0.06
Range	0.03 – 0.125	0.03 – 0.125	NA

*GM=Geometric Mean

The MIC distribution, MIC50, MIC90, geometric mean (GM) of Terbinafine for *T. mentagrophytes*, *T. rubrum* and *M. gypseum* is as shown in Table 3.

The MIC distribution, MIC50, MIC90, geometric mean (GM) of Itraconazole for *T. mentagrophytes*, *T. rubrum* and *M. gypseum* is as shown in Table 4.

The MIC distribution, MIC50, MIC90, geometric mean (GM) of Voriconazole for *T. mentagrophytes*, *T. rubrum* and *M. gypseum* is as shown in Table 5.

The results of micro dilution tests for most strains were read after 7 days at 28°C. Mean MICs of antifungal drugs did not show statistically significant differences between various species ($p>0.05$).

As can be seen in the tables above, the MIC50 and MIC90 values of Fluconazole (Table 1) and Griseofulvin (Table 2) for all three strains of dermatophytes isolated were found to be higher and those of Itraconazole (Table 4) and Voriconazole (Table 5) were found to be in the lower bracket and those for Terbinafine were found to lie in the intermediate range.

DISCUSSION

In our study, *Trichophyton* genus represented 97.6% of the isolates of dermatophytes, with *Trichophyton mentagrophytes* being the commonest that is 25 (60.98%), followed by *Trichophyton rubrum* 15 (36.58%) and *Microsporum gypseum* 1 (2.44%). Similar to our study, were the findings of the study conducted by Bhatia VK et al in the year 2014, in which *Trichophyton* species were implicated in 98.6% (73/74) cases while *Microsporum* species was detected only in 1.35% cases. Also, none of the *Epidermophyton* species was recovered by them. Further, *Trichophyton mentagrophyte* was also the predominant organism (64.9%) followed by *Trichophyton rubrum* (35.1%).[13] Trichophyton mentagrophyte was also the most common isolate in the study conducted by Sahai et al in the year 2011.[14] However, many studies have reported *Trichophyton rubrum* as the commonest isolate.[15-17]

Another important aspect of this study was to carry out antifungal sensitivity testing of five commonly used antifungal drugs Fluconazole, Terbinafine, Itraconazole, Griseofulvin and Voriconazole. Determining the resistance pattern is especially necessary to assist clinicians in treating superficial fungal infections more effectively.

The results of micro dilution tests for most strains were read after 7 days at 28°C, when adequate growth was observed in the control well with significant opacity. The 7 day time period has also been mentioned in the studies by Santos et al, Gupta et al, Fernandez-Torres et al and Barros et al.[18-21]

This time period was shorter (4 days at 35°C) in the study conducted by Ghannoum et al and Mukherjee et al.[22,23] This difference might be explained by the different temperatures used. Galuppi et al reported a longer period of 14 days and incubation at 30°C.[24] This difference in the required incubation time may be due to the different volumes of fungi inoculated into the micro plates.

Our findings about poor susceptibility of dermatophytes to Fluconazole (Table 1) is compatible with the studies conducted by Favre et al, Santos et al, Barros et al and Sarifakioglu et al.[18,21,25,26] Korting et al suggested that high values of MIC for Fluconazole may be due to technical problems, such as interference with some ingredients of the culture media or insolubility at high concentrations.[27] The easy availability of Fluconazole at pharmacies, self medication by patients due to its over the counter (OTC) preparations available and a rampant practice of its irrational prescription by quacks could be some other reasons for development of resistance to Fluconazole.

A high prevalence of resistance to Griseofulvin among dermatophytes (MIC range 0.03-16 µg/ml) as found in our study (Table 2) is in accordance with the findings of the studies by Galuppi et al and Korting et al.[24,27] For Griseofulvin, an MIC of 3 µg/ml was considered a limit of effectiveness.[24]

The geometric mean MIC (GM) obtained in this study (1.59 µg/ml) showed that the results of Terbinafine for all the three species of dermatophytes tested (Table 3) were significantly greater than the results obtained by Gupta et al (0.04 µg/ml), Favre et al (0.006 µg/ml), Deng et al (0.03 µg/ml), Esteban et al (0.03 µg/ml) and Fernandez-Torres et al (0.21 µg/ml) in their studies respectively.[19,25,28-30] This can be explained by a wide MIC range (0.03 - 16 µg/ml) found in our study probably due to a higher resistance of dermatophytes to Terbinafine in our area.

According to our study, Itraconazole (MIC range 0.03-0.125 µg/ml) and Voriconazole (MIC range 0.03-0.125 µg/ml) showed the lowest MIC ranges by the microbroth dilution technique which was also observed by Bueno et al in their study.[26] The high potency of Voriconazole and Itraconazole against dermatophytes are in accordance with the observations made by Favre et al in their study.[25]

The MIC range of Voriconazole found in our study (0.03-0.125 µg/ml) was found to correspond to the lower end of the MIC range found by Deng et al in their study (0.031-16 µg/ml).[28] An even lower MIC range (0.002 - 0.06 µg/ml) of Voriconazole was found by Ghannoum et al in their study.[31] The high sensitivity of dermatophytes to Voriconazole observed in our study can be attributed to the lower prevalence of its irrational prescription by quacks and chemists and also to its high cost.

The MIC range of Itraconazole (by microbroth dilution method) found in our study (0.03-0.125 µg/ml) was found to conform with the findings of Deng et al (0.031-16 µg/ml).[28]

In this study, Voriconazole was found to have the lowest geometric mean while Fluconazole had the highest geometric mean value and MIC range. Terbinafine was found to fall in the intermediate range. Therefore, it can be interpreted that Voriconazole being the most sensitive antifungal drug for dermatophytes is a more suitable treatment option but it must be reserved for resistant and difficult to treat cases so as to prevent rapid development of resistance. Itraconazole is a much more affordable antifungal drug that closely follows Voriconazole in its effectiveness against dermatophytes, hence, it must be a preferred treatment option for better outcome in patients suffering from dermatomycosis. Fluconazole being the

least sensitive antifungal drug against dermatophytes must be used cautiously due to its poor effect. The clinical significance of testing this group of fungi however remains uncertain, since relevant breakpoints are yet to be identified and approved by regulatory authorities. There is a need for establishing a standard method for antibiogram of dermatophytes to facilitate the selection of drug similar to what is routinely performed for yeasts (candida) and bacteria.

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