

IN VITRO ANTIFUNGAL SUSCEPTIBILITY OF GRISEOFULVIN, FLUCONAZOLE, ITRACONAZOLE AND TERBINAFINE AGAINST CLINICAL ISOLATES OF TRICHOPHYTON RUBRUM AND TRICHOPHYTON MENTAGROPHYTES

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ABSTRACT

Investigations on antifungal drug susceptibility were carried out on 90 clinical isolates of *Trichophyton rubrum*, and *Trichophyton mentagrophytes* with four antifungal drugs, namely griseofulvin, fluconazole, itraconazole and terbinafine as suggested by National Committee for Clinical Laboratory Standards (NCCLS) M27A (1997) document by broth macrodilution method to standardize in vitro antifungal susceptibility testing and to find out the Minimum Inhibitory Concentration (MIC) of the drugs. In this study, terbinafine was found to be the most efficient drug for all isolates. Terbinafine had the lowest MIC range of 0.001 g/ml to 0.09 g/ml and MIC₅₀ was low at 0.005 g/ml and MIC₉₀ was also low at 0.04 g/ml against *T. rubrum*; and MIC range of 0.001 µg/ml to 0.19 µg/ml with a MIC₅₀ of 0.01 µg/ml and MIC₉₀ at 0.09 µg/ml against *T. mentagrophytes*. Itraconazole showed antifungal activity superior to that of fluconazole, with a MIC range of 0.04 g/ml to 1.56 g/ml, with MIC₅₀ at 0.19 µg/ml and MIC₉₀ at 1.56 g/ml against *T. rubrum*; and MIC range of 0.04 µg/ml to 1.56 µg/ml, with MIC₅₀ at 0.19 µg/ml and MIC₉₀ at 0.78 µg/ml against *T. mentagrophytes*. Griseofulvin appears to be still a potent drug for management of dermatophytoses. Griseofulvin had a MIC range of 0.15 g/ml to 5.07 g/ml with MIC₅₀ at 1.26 g/ml and MIC₉₀ at 2.53 g/ml against *T. rubrum*; and MIC range of 0.31 µg/ml to 5.07 µg/ml with MIC₅₀ at 1.26 µg/ml and MIC₉₀ at 2.53 µg/ml against *T. mentagrophytes*. Fluconazole showed a high MIC range of 0.19 g/ml to 50 g/ml and MIC₅₀ was high at 1.56 g/ml and MIC₉₀ was also high at 12.5 g/ml against *T. rubrum*; and a high MIC range of 0.09 µg/ml to 25.0 µg/ml, with MIC₅₀ at 1.56 µg/ml and MIC₉₀ at 12.5 µg/ml towards *T. mentagrophytes*. The technique was found to be easy to perform and reliable with consistent results.

KEY WORDS: National Committee for Clinical Laboratory Standards (NCCLS), Antifungal drugs, Minimum Inhibitory Concentration (MIC), MIC₅₀, MIC₉₀, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, Griseofulvin, Fluconazole, Itraconazole, Terbinafine, Yeast Nitrogen broth, SDA.

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INTRODUCTION

Cases of dermatophytoses have increased over the past few decades. In the last few years, a number of newer less toxic antifungal drugs have become available for clinical use. The increased use of antifungals, often for prolonged periods, has led to the recognition of the phenomenon of acquired antifungal resistance¹ among previously susceptible strains or species and to the increased incidence of infections with less common species.

The rapid increase in fungal infections and the growing number of new antifungal agents² indicate an increasing need for rapid and accurate methods for antifungal susceptibility testing³. The present study describes and compares the in vitro susceptibility of clinical isolates of *T. rubrum*, and *T. mentagrophytes* against four antifungal drugs, namely to terbinafine⁴, itraconazole⁵, fluconazole⁶ and griseofulvin⁷.

MATERIALS AND METHODS

i. Isolation and Identification of the Isolates

A total of 90 isolates of *T. rubrum* and 37 isolates of *T. mentagrophytes* were obtained from 500 clinically diagnosed patients of tinea (ringworm) infection attending as out patients at Department of Skin & Venereology at Government General Hospital, Kolar, Karnataka during the period 2000-2004. They were identified by conventional morphological, cultural, and biochemical methods including urease test⁸, in vitro hair perforation test⁹, pigment test¹⁰, rice grain test¹⁰. The species isolated and tested (and numbers of isolated of each species) were as follows: *Trichophyton rubrum* (n = 90), *T. mentagrophytes* (n = 37).

ii. Antifungal Drugs

Four antifungal drugs namely griseofulvin, fluconazole, itraconazole and terbinafine were selected and tested for their activity. Griseofulvin stock solution was prepared in 70% ethanol, fluconazole in distilled water and itraconazole and terbinafine in dimethyl sulfoxide, that were stored at 20°C to 70°C. The concentrations in the stock solutions were 100 times the final concentration of each compound. Further dilutions of each antifungal agent were prepared using yeast nitrogen broth¹¹ as diluent.

The final concentrations ranged from 0.03 g/ml to 81.25 g/ml for griseofulvin; 0.047 g/ml to 50 g/ml for fluconazole; 0.02 g/ml to 25 g/ml for itraconazole and 0.0005 g/ml to 6.25 g/ml for terbinafine

Testing was performed by a broth macrodilution method¹² following the recommendation of the NCCLS M27A (1997). In brief, stock inocula of the *T. rubrum* and *T. mentagrophytes*

strains were prepared from 7 to 14 day cultures grown on Sabouraud's Dextrose Agar¹³ (SDA) with chloramphenicol. After the appearance of the sufficient growth 2 to 3 ml sterile normal saline (0.95%) was added and the suspensions were made by gently scraping the colony with the tip of a sterile Pasteur pipette. The resulting suspended mixture was withdrawn and transferred to a sterile tube. Heavy particles of the suspension, when present, were allowed to settle for 15 minutes at room temperature and the upper homogenous suspension was used for further testing. The suspensions were mixed with a vortex mixer for 15 seconds and adjusted with sterile normal saline to match an opacity of 0.5 McFarland's standard. The inoculum size was adjusted to between 1.0×10^6 and 5.0×10^6 spores/ml by microscopic enumeration with a cell counting haemocytometer (Neubauer chamber). In some instances where fungi do not readily produce conidia, small portion of the mycelial growth was harvested and gently homogenized in 2ml of sterile saline using tenbroeck tissue grinder and resulting suspensions were adjusted to an opacity of 0.5 McFarland standard¹⁴ by adding sterile saline. All standardized inocula were plated on SDA before the test to check the viability of the fungus. 0.3 ml of fungal inocula were added to the different drug dilutions. A control tube (broth without any drug but inoculated with the fungus) was included with each test. Tubes were incubated at 35° C in a BOD incubator until growth appeared in the drug free control tube. Incubation ranged 6 to 20 days. The highest dilution of the drug, which inhibited the fungal growth, was taken as the MIC. MIC₅₀ was calculated by taking the drug concentration, where fifty percent of isolates are inhibited. Similarly MIC₉₀ was noted with drug concentration where ninety percent of the isolates were inhibited.

RESULTS

The minimum inhibitory concentration¹⁵ (MIC₅₀ and MIC₉₀s) (Table1) of griesofulvin, fluconazole, itraconazole and terbinafine are compared to determine the efficacy and dosage of the drug for treatment of dermatophytoses.

Table 1: In vitro activities of four antifungal agents against 219 clinical isolates of dermatophytes

Species (No. of isolates)	MIC	Drug concentration μ(g/ml)			
		Griseofulvin	Fluconazole	Itraconazole	Terbinafine
<i>T. rubrum</i> (90)	Range	0.155-0.7	0.19-50.0	0.046-25	0.001- 0.09
	MIC ₅₀	1.26	1.56	0.19	0.005
	MIC ₉₀	2.53	12.5	1.56	0.04
<i>T. mentagrophyte</i> (37)	Range	0.31-5.07	0.09-25.0	0.041-1.56	0.001- 0.19
	MIC ₅₀	1.26	1.56	0.19	0.01
	MIC ₉₀	2.53	12.5	0.78	0.09

Griseofulvin exhibited MIC₅₀ at 1.26 g/ml for *T. rubrum*, and *T. mentagrophytes*. Fluconazole showed MIC₅₀ at 1.56 g/ml for *T. rubrum*, and *T. mentagrophytes*. Itraconazole showed MIC₅₀ at 0.19 g/ml for *T. rubrum*, and *T. mentagrophytes*. Terbinafine showed MIC₅₀ at 0.005 g/ml for *T. rubrum*, and 0.01 g/ml for *T. mentagrophytes*.

Griseofulvin exhibited MIC₉₀ at 2.53 g/ml against both *T. rubrum* and *T. mentagrophytes*. Fluconazole showed MIC₉₀ at 12.5 µg/ml for both *T. rubrum* and *T. mentagrophytes*. Itraconazole showed MIC₉₀ at 1.56 µg/ml for *T. rubrum* and 0.78 µg/ml towards *T. mentagrophytes*. Terbinafine showed MIC₉₀ at 0.04 µg/ml for *T. rubrum* and 0.09 µg/ml for *T. mentagrophytes*.

DISCUSSION

In the present study, antifungal susceptibility was carried out against 90 clinical isolates of *T. rubrum* and 37 isolates of *T. mentagrophytes* with four antifungal drugs, namely griseofulvin, fluconazole, itraconazole, and terbinafine as suggested by NCCLS M27-A (1997) document. In these investigations, terbinafine was found to be the most efficient drug for all the isolates of *T. rubrum* and *T. mentagrophytes*.

The MIC ranges of all the 90 isolates of *T. rubrum* and 37 isolates of *T. mentagrophytes* tested show that terbinafine had the lowest MIC range of 0.001 µg/ml to 0.09 µg/ml towards *T. rubrum* and 0.001 µg/ml to 0.19 µg/ml for *T. mentagrophytes*, followed by itraconazole with a MIC range of 0.04 µg/ml to 6.25 µg/ml for *T. rubrum* and 0.04 µg/ml to 1.56 µg/ml towards *T. mentagrophytes*. Griseofulvin showed a MIC range of 0.15 µg/ml to 5.07 µg/ml against *T. rubrum* and 0.31 µg/ml to 5.07 µg/ml towards *T. mentagrophytes*. Fluconazole showed a high MIC range of 0.19 µg/ml to 50.0 µg/ml towards *T. rubrum* and 0.09 µg/ml to 25.0 µg/ml against *T. mentagrophytes*. The MIC₅₀ of terbinafine was low at 0.005 µg/ml towards *T. rubrum* and 0.01 µg/ml for *T. mentagrophytes*; of itraconazole was 0.19 µg/ml for both *T. rubrum* as well as *T. mentagrophytes*; of griseofulvin was at 1.26 µg/ml for both *T. rubrum* and *T. mentagrophytes*; and of fluconazole was high at 1.56 µg/ml for both *T. rubrum* and *T. mentagrophytes*.

The MIC₅₀ of all the isolates tested show that terbinafine had the lowest at 0.005 µg/ml for *T. rubrum* and 0.01 µg/ml for *T. mentagrophytes*, followed by itraconazole at 0.19 µg/ml for both *T. rubrum* and *T. mentagrophytes*. Fluconazole showed a high MIC₅₀ at 1.56 µg/ml against both *T. rubrum* and *T. mentagrophytes*; of griseofulvin was at 1.26 µg/ml towards both *T. rubrum* and *T. mentagrophytes*.

The MIC₉₀ of terbinafine was low at 0.04 µg/ml for *T. rubrum* and 0.09 µg/ml for *T. mentagrophytes*; of itraconazole was at 1.56 µg/ml against *T. rubrum* and 0.78 µg/ml against *T. mentagrophytes*; of griseofulvin was at 2.53 µg/ml for both *T. rubrum* as well as *T. mentagrophytes*; and of fluconazole

was high at 12.5 µg/ml for both *T. rubrum* as well as *T. mentagrophytes*.

In the present study, terbinafine appears to be the most efficient drug for all the isolates of *T. rubrum* and *T. mentagrophytes*. Among the azoles, itraconazole was the second best drug against all *T. rubrum* and *T. mentagrophytes* isolates and found to be the better drug compared to fluconazole. Griseofulvin appears to be still a potent drug for the management of dermatophytoses caused by *T. rubrum* and *T. mentagrophytes*.

The present study indicates that the broth macrodilution method can be adopted for in vitro antifungal sensitivity testing, as it is simple, reproducible, cost effective and easy to perform in a routine clinical microbiology laboratory.

REFERENCES

- Alexander BD, Perfect JR. (2000) *Drugs* 54: 657-678
- Andriole VT, (2000) *Int. J. Antimicrob. Agents* 19: 317-322
- Cormican MG and Pfaller MA, (1993) *J. Antichemother.* 38: 561-578
- Gupta AK and Shear NH, (1997) *J. Am. Acad. Dermatol.* 37: 979-998
- Pfaller MA, Messer SA, Millis K and Bolmstoum A, (2000) *J. Clin. Microbiol.* 38: 3359-3361
- Agarwal PB, Narang A and Kumar P, (1996) *Indian J. Paediatr.* 63: 775-780
- Artis WM, Odle BM and Jones HE, (1981) *Arch. Dermatol.* 117: 16-19
- Bahuguna Sand Kushawaha RK, (1989) *Mycoses* 32: 340-343
- Chander J, (2002) *Text book of Medical Mycology, 2nd edition, Mehta publishers, New Delhi.*
- Jessup C, Warner J, Isham N, Hasan I and Ghannoum MA, (2000) *J. Clin. Microbiol.* 38: 341-344
- National Committee for Clinical Laboratory Standards (1997). *M27-A, NCCLS, Villanova, Pa*
- Norris HA, Elewski BE and Ghannoum MA, (1999) *J. Amer. Acad. Dermatol.* 40(6): S9-S13
- Espingel IA and Kerkering TM, (1991) *J. Clin. Microbiol.* 29: 393-394
- Fernandez TB, Carillo AJ, Martin E, Palacio AD, Moore MK, Valverde A, Serrano M and Guarrow J, (2001) *Antimicrob. Agents Chemother.* 45: 2524-2528
- Perea S, Fothergill AW, Sutton DA and Rinaldi MG, (2001) *J. Clin. Microbiol.* 39: 385-388