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) M27–A (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 0.001µg/ml to 0.19µg/ml with a MIC50 of 0.01µg/ml and MIC90 at 0.09µg/ml against T.mentagrophytes. Itraconazole showed antifungal activity superior to that of fluconazole, with a MIC range of 0.04g/ml to 1.56g/ml, with MIC50 at 0.19µg/ml and MIC90 at 1.56g/ml against T.rubrum; and MIC range of 0.04µg/ml to 1.56µg/ml, with MIC50 at 0.19µg/ml and MIC90 at 0.78µg/ml against T.mentagrophytes. Griseofulvin appears to be still a potent drug for management of dermatophytoses. Griseofulvin had a MIC range of 0.15g/ml to 5.07 g/ml with MIC50 at1.26 g/ml and MIC90 at 2.53 g/ml against T.rubrum; and MIC range of 0.31µg/ml to 5.07µg/ml with MIC50 at 1.26µg/ml and MIC90 at 2.53µg/ml against T.mentagrophytes. Fluconazole showed a high MIC range of 0.19 g/ml to 50 g/ml and MIC50 was high at 1.56g/ml and MIC90 was also high at 12.5 g/ml against T.rubrum; and a high MIC range of 0.09µg/ml to 25.0µg/ml, with MIC50 at 1.56µg/ml and MIC90 at 12.5µg/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.

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 resistance[1] 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 agents[2] indicate an increasing need for rapid and accurate methods for antifungal susceptibility testing[3], 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 terbinafine[4], itraconazole[5] fluconazole[6] and griseofulvin[7].

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 test[8], in vitro hair perforation test[9], pigment test[10], rice grain test[10]. The species isolated and tested (and numbers of isolated of each species) were as follows: Trichophyton rubrum (n = 90), T. mentagrophytes (n = 37).

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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 –20oC to –70oC. 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 broth[11] 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 method[12] following the recommendation of the NCCLS M27–A (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 agar[13] (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 x 106 and 5.0 x 106 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 35o 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 (Minimum Inhibitory Concentration). 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, itraconzole and terbinafine are compared to determine the efficacy and dosage of the drug for treatment of dermatophytoses.

Griseofulvin (Text fig 1) exhibited MIC50 at 1.26 g/ml for T. rubrum, and T. mentagrophytes. Fluconazole (Text fig 2) showed MIC50 at 1.56 g/ml for T. ruburm, and T. mentagrophytes. Itraconazole (Text fig 3) showed MIC50 at 0.19 g/ml for T. rubrum, and T. mentagrophytes. Terbinafine (Text fig 4) showed MIC50 at 0.005 g/ml for T. rubrum, and 0.01 g/ml for T. mentagrophytes.

Grisefulvin16 exhibited MIC90 at 2.53 g/ml against both T. rubrum and T.mentagrophytes. Fluconazole showed MIC90 at 12.5µg/ml for both T.rubrum and T.mentagrophytes. Itraconazole showed MIC90 at 1.56µg/ml for T.rubrum and 0.78µg/ml towards T.mentagrophytes. Terbinafine showed MIC90 at 0.04µg/ml for T.rubrum and 0.09µg/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 0.001µg/ml to 0.09µg/ml towards T.rubrum and 0.001µg/ml to 0.19µg/ml for T.mentagrophytes, followed by itraconazole with a MIC range of 0.04µg/ml to 6.25µg/ml for T.rubrum and 0.04µg/ml to 1.56µg/ml towards T.mentagrophytes. Griseofulvin showed a MIC range of 0.15µg/ml to 5.07µg/ml against T.rubrum and 0.31µg/ml to 5.07µg/ml towards T.mentagrophytes. Fluconazole showed a high MIC range of 0.19µg/ml to 50.0µg/ml towards T.rubrum and 0.09µg/ml to 25.0µg/ml against T.mentagrophytes. The MIC50 of terbinafine was low at 0.005µg/ml towards T.rubrum and 0.01µg/ml for T.mentagrophytes; of itraconazole was

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

Species		Drug concentration (? g/ml)				
(No. of isolates)	MIC	Griseofulvin	Fluconazole	Itraconazole	Terbinafine	
T.rubrum (90)	Range	0.15-5.07	0.19-50.0	0.04-6.25	0.001 - 0.09	
	MIC_{50}	1.26	1.56	0.19	0.005	
	MIC_{90}	2.53	12.5	1.56	0.04	
T. mentagrophytes (37)	Range	0.31-5.07	0.09-25.0	0.04-1.56	0.001 - 0.19	
	MIC_{50}	1.26	1.56	0.19	0.01	
	MIC_{90}	2.53	12.5	0.78	0.09	

0.19µg/ml for both T.rubrum as well as T.mentagrophytes; of griseofulvin was at 1.26µg/ml for both T.rubrum and T.mentagrophytes; and of fluconazole was high at 1.56µg/ml for both T.rubrum and T.mentagrophytes.

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

The MIC90 of terbinafine was low at 0.04µg/ml for T.rubrum and 0.09µg/ml for T. mentagrophytes; of itraconazole was at 1.56µg/ml against T.rubrum and 0.78µg/ml against T.mentagrophytes;of griseofulvin was at 2.53µg/ml for both T.rubrum as well as T.mentagrophytes; and of fluconazole was high at 12.5µg/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.

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