



## Research Article

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# Antifungal susceptibility testing for dermatophytes isolated from clinical samples by broth dilution method in a tertiary care hospital

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## ABSTRACT

**Purpose:** To characterize antifungal susceptibility pattern of different species of dermatophytes isolated from clinical patients in a multispeciality hospital. **Method:** Micro broth dilution method was used to determine Minimum inhibitory concentration (MIC) for 129 positive samples with reference to CLSI document M38-A2. MIC was done against amphotericin B, fluconazole, ketoconazole, terbinafine, ciclopirox and griseofulvin. **Results:** The range of MIC was within the normal susceptibility range of the standard strain (*Trichophyton mentagrophytes* ATCC MYA – 4439) mentioned in CLSI document M-38 A2. **Conclusion:** No resistant strains were isolated in our study with reference to the standard testing method. None of the isolates have showed abnormal MIC range when compared to MIC of standard strain reported in literature. Hence, the isolates in our study are found to be susceptible to the antifungals used. However, future studies are required to evaluate the effect of drugs upon clinical response.

**Keywords:** Dermatophytes, Antifungal susceptibility testing, Minimum inhibitory concentration.

## INTRODUCTION

The increasing incidence and prevalence of fungal infection in developing countries is attributed to immunocompromised state such as use of corticosteroids, immunosuppressive agents, anticancer drugs, HIV-positivity, etc. Most infections of skin and its appendages, the hair and nail are caused by a homogenous group of keratinophilic fungi called the dermatophytes<sup>[1]</sup>. The members of this dermatophytic group include *Trichophyton*, *Microsporum* and *Epidermophyton*<sup>[2]</sup>.

The various antifungal agents now available for clinical use against dermatophytes are terbinafine, itraconazole, fluconazole, ketoconazole and voriconazole. However, their activity against different species of dermatophyte has not yet been fully investigated<sup>[3]</sup>. Development of resistant strains will results due to inappropriate use of antifungal agents<sup>[4]</sup>.

Clinical and Laboratory Standards Institute (CLSI) had developed the standard broth micro dilution M38-A2 method for antifungal susceptibility of some filamentous fungi, including the dermatophytes in 2008<sup>[1, 5]</sup>. Various studies with variable results were reported for antifungal susceptibility testing of dermatophytes. In developing a standardized method for antifungal susceptibility testing of dermatophytes several variables need to be considered, like the medium for conidiation, the size of inoculum, temperature and duration of incubation, medium of inoculation, and endpoint determination. The purpose of this study is to perform in vitro antifungal susceptibility using broth micro-dilution method (CLSI M38-A2) and to find out the MIC range of isolated dermatophytes for amphotericin B, terbinafine, ciclopirox, ketoconazole, fluconazole & griseofulvin.

## MATERIALS AND METHODS

Human ethical clearance for the study was obtained from the Institutional ethical committee prior to the collection of samples. A total of 300 (skin, nail & hair) specimens were collected from the clinically suspected cases of dermatophytic infections for the period of twelve months after informed consent. About 129 samples were found to be positive for dermatophytes by macroscopic and microscopic morphology, slide culture, hair perforation test, urease test and growth characters on Bromocresol Purple agar (BCP).

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### Antifungal susceptibility testing

The aim of doing antifungal susceptibility testing is to find the Minimum Inhibitory concentration (MIC) and the antifungal agents used to find the MIC for dermatophytes are amphotericin B (Himedia), fluconazole (Himedia), ketoconazole (Himedia), ciclopirox (Sigma Aldrich), terbinafine (Sigma Aldrich) and griseofulvin (Sigma Aldrich). The stock solutions for the antifungal agents were prepared. Rose Parker Memorial Institute – 1640 (RPMI-1640) is used as a growth medium in antifungal susceptibility testing (pH - 7.0 ± 0.1). The isolated dermatophytic colony to be tested is grown in potato dextrose agar (PDA) for conidia formation. After required growth, the conidia's were taken in a concentration of 1 – 3 X 10<sup>3</sup> cfu/ml and are inoculated in sterile 96 – well microtiter plate with flat bottom. Each well is inoculated with 100 µl of the conidial suspension in RPMI 1640 and 100µl of diluted drugs are added correspondingly to each well. The growth control well and sterility control well were also added. All microtitre plates are incubated at 37°C for four days. *Trichophyton mentagrophytes* ATCC MYA – 4439 is used as the control.

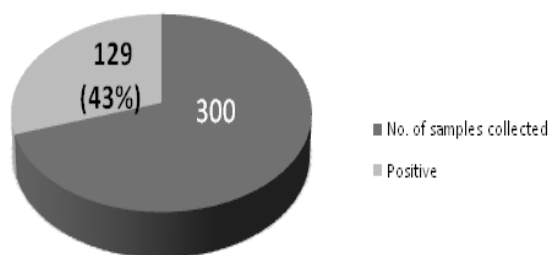


Figure 1: Percentage of samples positive by culture in our study

### DISCUSSION

Dermatophytes are group of fungal agents causing infection of skin, hair and nail. The reason for treatment failure and development of resistance is attributed to decreased drug uptake, phenotypic or genotypic alterations or increase in drug efflux.

The present work is conducted to determine the antifungal spectrum of dermatophytes in our region. Dermatophytes were grown in 129 (43%) samples out of 300 samples collected in our study. The antifungal susceptibility was done by broth dilution method with references to CLSI document M38-A2<sup>[1]</sup>. The drugs evaluated in this study are amphotericin B, fluconazole, ketoconazole, ciclopirox, terbinafine and griseofulvin. *Trichophyton mentagrophytes* ATCC MYA – 4439 is used as the control in performing the antifungal susceptibility testings. The antifungal susceptibility testing for dermatophytes was done by Fernandez *et al*<sup>[5, 6]</sup>, Ghannoum *et al*<sup>[7]</sup>, Jessup *et al*<sup>[8]</sup> with various antifungal drugs. Clinically confirmed cases of drug resistance for *Trichophyton rubrum* to terbinafine, azoles and griseofulvin were reported by Osborne *et al*<sup>[9]</sup> and Mukherjee *et al*<sup>[10]</sup>.

The results of antifungal susceptibility testing for eight different species (*Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton ajelloi*, *Trichophyton violaceum*, *Microsporum gypseum*, *Microsporum ferrugineum* and *Epidermophyton floccosum*) in

For amphotericin B end points are typically well defined and the MIC is easily read as the lowest drug concentration that prevents any discernible growth (100%). For all other drugs (fluconazole, ketoconazole, ciclopirox, griseofulvin and terbinafine) end points are not typically as well defined as that of amphotericin B. Usually it is taken as 80% or more reduction in growth when compared to growth in control well (drug free medium).

### RESULTS

A total of 300 samples were collected from the patients including skin, hair and nail. Out of which 129 (43%) samples were found to be positive for dermatophytes (Figure 1). The majority of the isolates were *Trichophyton mentagrophytes* followed by *Trichophyton rubrum*. Various species isolated were shown in Figure 2. The antifungal susceptibility testing were done for 129 dermatophytes by broth micro dilution method. The range of MIC was within the normal susceptibility range of the standard ATCC fungal strains mentioned in CLSI document M-38 A2. The results of antifungal susceptibility testing were shown in Table 1 for all the species isolated in our study.

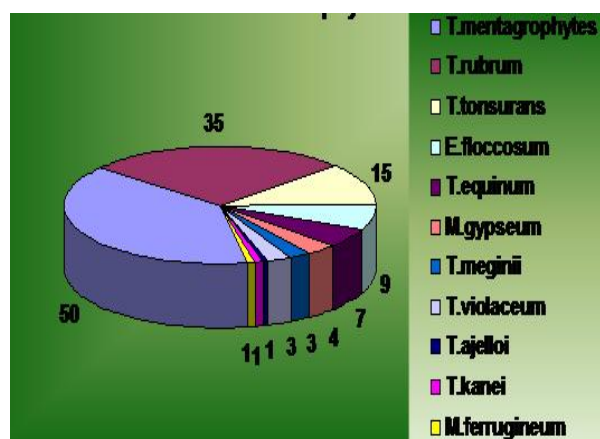


Figure 2: Spectrum of dermatophytes isolated from the samples in our study

our study correlated well with the results obtained by previous studies except for the results of Amphotericin B. Higher MIC values are obtained for Amphotericin B in our study for *Trichophyton mentagrophytes* (0.5 – 8 µg/ml), and *Trichophyton tonsurans* (1 – 8 µg/ml). Whereas, the studies by Fernandez *et al*<sup>7</sup> shows the MIC range for *Trichophyton mentagrophytes* and *Trichophyton tonsurans* as 0.125 - 1 µg/ml and 0.03 – 0.5 µg/ml respectively. However, the patients were not treated with Amphotericin B to know the clinical outcome.

No previous studies were available for antifungal susceptibility of *Trichophyton equinum*, *Trichophyton meginii* and *Trichophyton kanei*. But we have done MIC for these species also and the results were shown in Table 1. No resistant strains were isolated in our study with reference to the standard testing method. There are no significant differences of MIC between the species when compared to the previous studies (P<0.01). The management of dermatophytic infections needs personal hygiene, awareness of infection, proper diagnosis and appropriate medication. The clinical response of the patients decides the susceptible nature of drugs, rather than in vitro testing's. A standard reference method for the testing of the antifungal susceptibilities of dermatophytes and prevent the emergence of resistance is very important.

**Table 1:** Showing the MIC pattern of 129 isolates of dermatophytes obtained from the study

Drugs	Amphotericin B (0.0313 - 16µg/ml)		Fluconazole (64- 0.125µg/ml)		Ketoconazole (16- 0.0313µg/ml)		Ciclopirox (32- 0.06µg/ml)		Terbinafine (0.5-0.001µg/ml)		Griseofulvin (16-0.0313µg/ml)	
Isolates	MIC range (µg/ml)	Median	MIC range (µg/ml)	Median	MIC range (µg/ml)	Median	MIC range (µg/ml)	Median	MIC range (µg/ml)	Median	MIC range (µg/ml)	Median
<i>Trichophyton mentagrophytes</i>	0.5 - 8	4	1 - 8	2	0.0313-1	0.5	0.125-2	1	0.001-0.008	0.004	0.25-0.5	0.5
<i>Trichophyton rubrum</i>	2 - 8	8	0.125 - 2	2	0.25 - 1	0.5	0.125-2	1	0.001-0.008	0.004	0.25-0.5	0.5
<i>Trichophyton tonsurans</i>	1 - 8	4	1-4	2	0.313 - 0.25	0.25	1-2	1	0.001-0.004	0.004	0.125-0.5	0.125
<i>Trichophyton equinum</i>	4 - 8	4	2	2	0.25	0.25	0.5-1	1	0.004	0.004	0.25	0.25
<i>Trichophyton meginii</i>	4 - 8	4	0.25-0.5	0.5	0.25	0.25	2	2	0.004	0.004	0.25	0.25
<i>Trichophyton ajelloi</i>	4	4	1	1	1	1	0.5	0.5	0.002	0.002	0.5	0.5
<i>Trichophyton violaceum</i>	2 - 4	2	0.25-0.5	0.5	0.25-1	1	1	1	0.004	0.004	0.5	0.5
<i>Trichophyton kanei</i>	4	4	4	4	0.5	0.5	1	1	0.004	0.004	0.5	0.5
<i>Microsporum gypseum</i>	0.5 - 2	0.5	2-4	4	0.25-0.5	0.25	0.125-1	1	0.004	0.004	0.125-0.5	0.5
<i>Microsporum ferrugineum</i>	4	4	8	8	1	1	2	2	0.008	0.008	0.5	0.5
<i>Epidermophyton floccosum</i>	2 - 4	2	1-2	2	1-2	2	0.5-1	1	0.001-0.004	0.004	0.25-0.5	0.5
<i>Trichophyton mentagrophytes</i> ATCC MYA- 4439	-	-	-	-	-	-	0.5-2	1	0.002- 0.008	0.004	0.12-0.5	0.25

## CONCLUSION

Dermatophytes are the most common cause of infectious skin disease. The most challenging task is not the diagnosis but the treatment and patient recovery. Since it requires a long time treatment, the management with appropriate and responsive drug is the need of this era. Hence it's the role of microbiology lab to provide the clinician with reliable diagnosis to aid their treatment. Future studies are needed which helps in rapid diagnosis and drug susceptibility.

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**Conflict of Interest:** No conflict of interest noted. No financial implication.

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