



## Original Research Article

## In vitro MIC comparison of a test itraconazole brand versus 4 brands of itraconazole against clinical isolates of *Trichophyton mentagrophytes* and *Trichophyton rubrum* using broth dilution method and disc diffusion method

Bela Nabar<sup>1</sup>, Swapnil Lokegaonkar<sup>1,\*</sup>, Rohan Mohan<sup>1</sup>

<sup>1</sup>Smt Chandibai Himathmal Mansukhani College, Ulhasnagar, Maharashtra, India



## ARTICLE INFO

## Article history:

Received 26-02-2020

Accepted 04-03-2020

Available online 21-04-2020

## Keywords:

MIC

Itraconazole

*Trichophyton mentagrophytes*

*Trichophyton rubrum*

broth dilution

## ABSTRACT

Dermatophytosis is one of the most common cutaneous fungal infections in India. Despite, quite a few numbers of antifungal agents available for treatment, the resistance to antifungals by dermatophytes is increasing day by day as seen by doctors in clinical practice. Itraconazole has shown good results in the treatment of dermatophytosis at doses of 100 mg once a day for 2 weeks and with 200 mg once a day for 7 days. However, there are multiple itraconazole brands available in India. In this study, five different itraconazole brands were evaluated in vitro for MIC values against clinical isolates of *Trichophyton mentagrophytes* and *Trichophyton rubrum* by two different methods i.e. broth dilution and disc diffusion tests. It was found that the Reference brand of itraconazole and the test brand of Itraconazole showed least MIC values as compared to other available brands.

© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by/4.0/>)

## 1. Introduction

Infection of the hair, skin, or nails caused by a fungus dermatophyte is called as dermatophytosis. Amongst dermatophytes, the most common genus is *Trichophyton* followed by *Microsporum* or *Epidermophyton* genera. *Tinea capitis*, *tinea pedis*, and *onychomycosis* are common dermatologic diseases caused by dermatophytes.<sup>1</sup> *Trichophyton rubrum* and *Trichophyton mentagrophytes*, which cause infections of skin and nails, are two of the most commonly isolated dermatophytes.<sup>2</sup>

A large number of safe and effective antifungal agents are available for the treatment of dermatophytosis.<sup>3</sup> Antifungal agents such as triazoles (itraconazole, fluconazole), imidazole (ketoconazole), allylamine (terbinafine) and griseofulvin have been reported to have substantial activity in dermatophytosis.<sup>4</sup>

It has been observed recently that there has been widespread resistance to various antifungal agents used

in conventional dose with an increase in relapse rates prompting a need to find an effective first-line antifungal drug and appropriate dosage and duration schedule to achieve maximum results with fewer relapses. Itraconazole has shown good results in the treatment of dermatophytosis at doses of 100 mg once a day for 2 weeks and with 200 mg once a day for 7 days.<sup>5</sup>

In India, there are multiple itraconazole brands including innovator are available for the treatment of dermatophytosis. The test brand of itraconazole, used for the study was launched in India since 2012. The purpose of the present study was to evaluate in vitro susceptibility of the test brand of Itraconazole vs 4 other itraconazole brands available in India including reference brand. A standard method for susceptibility testing of dermatophytes is lacking, but good results of MIC using either broth dilution or disc diffusion tests have been obtained in several papers.

\* Corresponding author.

E-mail address: dr.roshan.gmc@gmail.com (S. Lokegaonkar).

## 2. Material and Methods

### 2.1. Specimen

The strains of *T. mentagrophytes* and *T. rubrum* (50 of each) used in this study were obtained from skin and nails of patients with dermatophytosis and onychomycosis. The isolates were identified by routine mycological procedures and were maintained in sterile distilled water at 4°C until tests were performed.

### 2.2. Itraconazole dilutions

The Itraconazole was obtained from all 5 manufacturers including innovator. Drugs were dissolved in 100% dimethyl sulfoxide (Sigma-Aldrich). They were subsequently prepared as stock solution and serial twofold dilutions were performed.

### 2.3. Broth dilution method

The Dermatophytes were sub-cultured on Sabouraud Dextrose Agar & incubated at 28°C for 9-10 days to enhance sporulation. The growth was harvested in sterile saline & the conidial and hyphal suspension was adjusted to 1x10<sup>6</sup>/ml using a haemocytometer. This suspension is used as an inoculum for the broth dilution method.

For the broth the dilution sterile Sabouraud Dextrose broth supplemented with 2% Glutamine was used. The stock solution with 20 µg/ml (w/v) of each formulation was prepared. Stock solution was then used to perform 2-fold serial dilution to achieve the concentration range of 10 µg/ml to 0.005 µg/ml (v/v). Tubes were then inoculated with 0.1 ml of inoculum and incubated at 28°C for 9-10 days. The medium control and Positive Growth control of inoculum was also maintained along with the test sets. After the completion of incubation period the tubes were observed for turbidity.

### 2.4. Agar Disc Diffusion Method

The Dermatophytes were sub-cultured on Sabouraud Dextrose Agar & incubated at 28°C for 9-10 days to enhance sporulation. The growth was harvested in sterile saline & the conidial and hyphal suspension was adjusted to 1x10<sup>6</sup>/ml using a haemocytometer. This suspension is used as an inoculum for the Agar Disk Diffusion method.

The Sabouraud Dextrose Agar supplemented with the 2% Glutamine were used to perform the test. The 10 µg/µl, 2.5 µg/µl, 0.18 µg/µl, 0.04 µg/µl and 0.01 µg/µl concentrations of formulations were made in filter sterile DMSO. 5 mm filter paper disks were made, autoclaved and dried. Sabouraud agar plates with 2% Glutamine were inoculated with the dermatophytes using sterile swab and formulation disc were placed on the agar plates. The plates were sealed with parafilm and kept for incubation at 28°C for 9-10 days. After the completion of incubation plates

were observed for zone of inhibition. The outer diameter of each such zone measured and results were interpreted accordingly.

### 2.5. Endpoint determination

For broth dilution method, the tube with least concentration of formulation showing no turbidity was considered as MIC. The medium control tube did not show any turbidity while Positive Growth Control tubes exhibited turbidity indicating growth of the inoculum.

## 3. Results

MICs of antifungal agents for dermatophytes isolates could be determined after four days for *T. mentagrophytes* and five days for *T. rubrum* when incubated at 28°C.

ummarizes the MIC ranges for all brands against the isolates of *T. mentagrophytes* and ummarises MIC ranges against *T. rubrum*.

**Table 1:** Minimum inhibitory concentration of test formulations against *T. mentagrophytes* (Broth dilution method)

No	Formulation	Minimum Inhibitory concentration (µg/ml)
1	Test brand	0.35
2	Reference brand	0.18
3	Brand A	0.67
4	Brand B	10
5	Brand C	10

**Table 2:** Minimum inhibitory concentration of test formulations against *T. rubrum* (Broth dilution method)

No	Formulation	Minimum Inhibitory concentration (µg/ml)
1	Test brand	1.25
2	Reference brand	0.35
3	Brand A	1.25
4	Brand B	10
5	Brand C	10

For agar disc diffusion method, diameter of zone of inhibition are illustrated in Tables 3 and 4 for *T. mentagrophytes* and *T. rubrum* respectively.

## 4. Discussion

Dermatophyte infections are widespread and cause significant distress to the patients socially, emotionally and financially. Resistant dermatophytosis is fast emerging as a challenge for dermatologists in India. *T. mentagrophytes* is one of the most commonly isolated organisms. This has been the most frequently isolated organism in some reports from India, although in some studies of *tinea corporis* and *T. cruris*, *T. rubrum* has been more frequently isolated.<sup>6</sup>

**Table 3:** Effect of test formulation on the growth of *Trichophyton mentagrophytes* (Agar disc diffusion method)

No	Concentration (µg)	Diameter of Zone of Inhibition (mm)				
		Test brand	Reference brand	Brand A	Brand B	Brand C
1	10	35	36	35	34	34
2	2.5	33	32	31	35	32
3	0.18	30	26	24	24	28
4	0.04	25	25	15	21	20
5	0.01	21	15	8	9	15

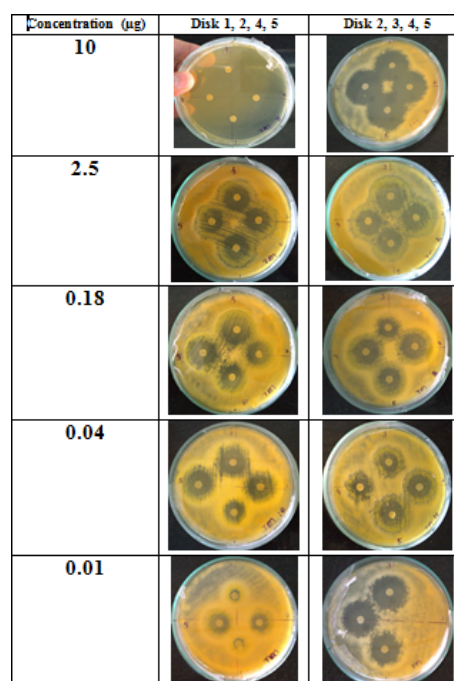
**Table 4:** Effect of test formulation on the growth of *Trichophyton Rubrum* (Agar disc diffusion method)

No	Concentration (µg)	Diameter of Zone of Inhibition (mm)				
		Test brand	Reference brand	Brand A	Brand B	Brand C
1	10	35	35	32	30	30
2	2.5	28	30	28	28	25
3	0.18	24	28	24	26	20
4	0.04	25	26	15	14	18
5	0.01	24	15	13	NI	10

Widespread resistance to conventional doses of antifungals with increasing clinical failure rates warrants the search for an effective first-line antifungal drug that brings about rapid clinical and mycological cure in tinea corporis and tinea cruris. Itraconazole is a triazole antifungal drug which is also increasingly being used as a first-line drug for tinea corporis and tinea cruris. In a study, Itraconazole was the most effective antifungal agent against *T. mentagrophytes*, *T. rubrum*.<sup>7</sup>

The taxonomy of dermatophytes is evolving with increased use of molecular techniques and *T. mentagrophytes* and *T. rubrum* are now considered as a species complex with many species defined within each species.<sup>8</sup> In this study, a CLSI protocol (standard M38- A) adapted was followed to determine the MIC values of five different brands of itraconazole currently employed for management of dermatophytosis in India. The determination of the in vitro susceptibility may prove helpful to predict the ability of a given antifungal agent to eradicate dermatophytes. Although a reference method for dermatophytes, is not available, a good correlation between the in vitro data, using broth microdilution method or agar disc diffusion method, and clinical outcome has been demonstrated.<sup>9</sup>

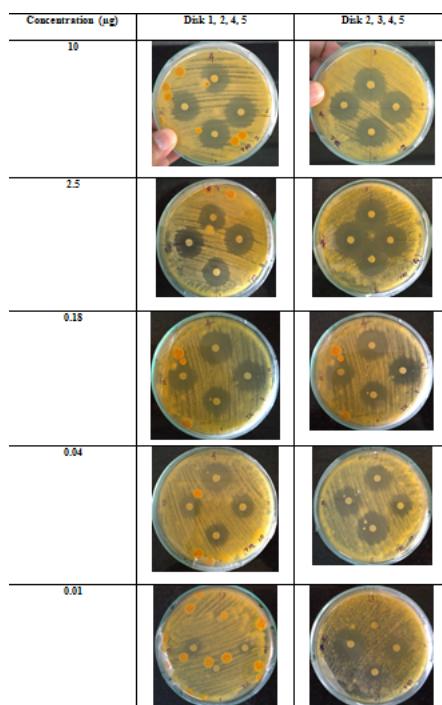
With broth dilution method, Reference brand of itraconazole showed least MIC against *T. mentagrophytes* (0.18 µg/ml) and *T. rubrum* (0.35 µg/ml). The test Itraconazole Formulation was found to be the closest to Reference itraconazole brand with respect to MIC concentration among other four Formulations. The MIC of test Itraconazole brand for *T. mentagrophytes* and *T. rubrum* were 0.35 µg/ml and 1.25 µg/ml respectively. In case of agar disc diffusion method, among 5 tested formulations the zone of inhibition pattern of Test Itraconazole brand and Reference itraconazole brand found to be similar against *T. rubrum* and *T. mentagrophytes*.



**Fig. 1: Name of the test:** Agar disk diffusion assay  
**Test Pathogen:** *Trichophyton mentagrophytes* Clinical Isolate  
**Key:**  
**Disk 1:** Brand A 100, **Disk 2:** Brand C 100, **Disk 3:** Brand B 100,  
**Disk 4:** Test Itraconazole brand, **Disk 5:** Reference brand

### 5. Conclusion

This is the first of its kind study where different antifungal preparations of same molecule i.e. itraconazole were evaluated using two different in vitro methods. It can be concluded that MIC values for Test Itraconazole brand and reference itraconazole brands were least as compared



**Fig. 2: Name of the test:** Agar disk diffusion assay **Test Pathogen:** *Trichophyton rubrum* Clinical Isolate  
**Key:**  
**Disk 1:** Brand A 100, **Disk 2:** Brand C 100, **Disk 3:** Brand B 100,  
**Disk 4:** Test Itraconazole brand, **Disk 5:** Reference brand

to other generic brands when assessed using in vitro susceptibility testing including broth solution and agar disc diffusion methods against the clinical isolated of *T. rubrum* and *T. mentagrophytes*

## 6. Acknowledgements

We acknowledge our sincere gratitude to Glenmark Pharmaceuticals Limited for help in data analysis and preparation of the manuscript.

## 7. Source of funding

This study was carried out with the grant from Glenmark Pharmaceuticals Limited, as an unrestricted educational grant.

## 8. Disclaimer and Conflict of interest

The authors declare that they have no conflict of interest. The design or procedure of the study and the content of the paper are in no way influenced by the grant provider.

## References

- Gupta AK, Rosso JQD, and. An evaluation of intermittent therapies used to treat onychomycosis and other dermatomycoses with the oral antifungal agents. *Int J Dermatol.* 2000;39(6):401–411.
- M EF, Tietz HJ, Presber W, Sterry W, Gräser Y. Development of an oligonucleotide probe specific for *Trichophyton rubrum*. *Br J Dermatol.* 1999;141:240–245.
- Favre B, Hofbauer B, Hilderling KS, Ryder NS. Comparison of In Vitro Activities of 17 Antifungal Drugs against a Panel of 20 Dermatophytes by Using a Microdilution Assay. *J Clin Microbiol.* 2003;41(10):4817–4819.
- Gupta AK, Rosso JQD, and. An evaluation of intermittent therapies used to treat onychomycosis and other dermatomycoses with the oral antifungal agents. *Int J Dermatol.* 2000;39(6):401–411.
- Bhatia A, Kanish B, Badyal D, Kate P, Choudhary S. Efficacy of oral terbinafine versus itraconazole in treatment of dermatophytic infection of skin – A prospective, randomized comparative study. *Indian J Pharmacol.* 2019;51:116–119.
- Dogra S, Pathania S, Rudramurthy S, Narang T, Saikia U. A prospective study of the epidemiological and clinical patterns of recurrent dermatophytosis at a tertiary care hospital in India. *Indian J Dermatol, Venereol Leprol.* 2018;84(6):678–684.
- Ganeshkumar P, Mohan S, Hemamalini M, Madhavan R, Lakshmanan A. Epidemiological and clinical pattern of dermatomycoses in rural India. *Indian J Med Microbiol.* 2015;33(5):134–136.
- Jensen RH, Arendrup MC. Molecular diagnosis of dermatophyte infections. *Curr Opin Infect Dis.* 2012;25(2):126–134.
- Korting HC, Ollert M, Abeck D. Results of German multicenter study of antimicrobial susceptibilities of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains causing tinea unguium. German Collaborative Dermatophyte Drug Susceptibility Study Group. *Antimicrob Agents Chemothe.* 1995;39(5):1206–1208.

## Author biography

Bela Nabar HOD

Swapnil Lokegaonkar Student

Rohan Mohan Student

**Cite this article:** Nabar B, Lokegaonkar S, Mohan R. **In vitro MIC comparison of a test itraconazole brand versus 4 brands of itraconazole against clinical isolates of *Trichophyton mentagrophytes* and *Trichophyton rubrum* using broth dilution method and disc diffusion method.** *IP Indian J Clin Exp Dermatol* 2020;6(1):67-70.