

# Evaluation of in Vitro Antifungal Activity of Ketoconazole and Griseofulvin

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## Abstract

Antifungal activity of Ketoconazole and Griseofulvin was evaluated on 36 isolates of different strains of dermatophytes and yeasts, and the results were compared.

Ketoconazole had a broad spectrum of antifungal activity although not all the strains were sensitive to it, yet it was much superior in its activity to Griseofulvin (JPMA 33:230, 1983).

## Introduction

Ketoconazole, which is one of the newer imidazole antifungal agents, has an in vitro wide spectrum of antifungal activity, which includes dermatophytes, yeasts, dimorphic fungi, eumycetes, actinomycetes, some phycomyces and other fungi (Heel et al., 1982).

Moreover it is the most useful of the imidazole compounds, since it can be given by mouth.

As only a very limited data is available comparing Ketoconazole with Griseofulvin, the other oral antifungal agent, this study was undertaken to find the antifungal spectrum of Ketoconazole against the local isolates and compare its activity with Griseofulvin, and also to find the incidence of primary fungal resistance to Ketoconazole, as the drug has not yet been introduced in this country.

## Material and Methods

### Fungi:

The dermatophytes and the yeasts tested were isolated from the clinical material in the Pathology Department of Khyber Medical College, Peshawar.

The method of testing was modified from that of Zimmerman (1979).

### Drugs:

Ketoconazole and Griseofulvin were dissolved in 95 percent ethanol and diluted in aqueous 0.9 percent NaCl to a final dilution of 10 mcg per ml.

The blood concentration of Ketoconazole after a single dose of 200 to 400 mg. per day reaches 2 and 3 mcg. per ml. respectively after 4 hours (Craven et al., 1982); and after a single dose of 0.5 to 1 gm. of micronised Griseofulvin reaches 1 to 3 mcg/ml. (Wade and Reynolds, 1977).

### Test:

The fungus was grown at 28 degrees centigrades for 3 weeks. The mycelium was washed off with sterile 0.9 percent NaCl and 0.1 ml. of this suspension was smeared on nutrient agar plates, which had previously been smeared with Kanamycin 30 mcg and Actidione 50 mcg in 0.1 ml. of normal saline. The plates were then dried. The tests were done in duplicate.

Holes (5 mm diameter) were punched in the plates and 0.1 ml. of solutions of Ketoconazole, Griseofulvin and 0.7 percent alcohol (as control) were pipetted into the holes.

### Incubation:

The plates were incubated at 28 degrees centigrade until aerial mycelium appeared in dermatophytes or growth in yeasts.

### Interpretation of results:

The inhibition zones were measured in millimeters. The interpretive criteria used for Ketoconazole were Sensitive: Inhibitory zone more than 20mm -MIC less than 1 mcg per ml.

Intermediate: Inhibitory zone size between 12-19 mm MIC 1-4 mcg per ml.

Resistant: Inhibitory zone size less than 12 mm MIC more than 4 mcg per ml. (Shadomy and Espinola-Ingroff, 1980). Criteria used for Griseofulvin were the same.

## Result

Antifungal activity of Ketoconazole was evaluated on 36 isolates of dermatophytes and *Candida* spp. and the results were compared with those of Griseofulvin.

The isolates tested were *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Trichophyton schoenleini*, *Microsporum ferrugineum*, *Trichophyton violaceum*, *Trichophyton tonsurans* and *Candida* spp. (Table).

Antifungal Activity of Ketoconazole Compared to Griseofulvin

Species	Number of Strains tested	Number of Strains resistant	number of Strains intermediate	Number of Strains sensitive	diameter of inhibition zone in millimeters (average of two readings)			Remarks
					K/G	K/G	K/G	
<i>Micro. ferrugineum</i>	8	6/8	2/0	0/0	14 & 15	0	0	
<i>Tricho. schoenleini</i>	10	1/6	0/0	9/4	21 to 38	25 to 36	0	
<i>Tricho. mentagrophytes</i>	5	5/5	0/0	0/0	0	0	0	in two cases growth was present even in the punched holes.
<i>Tricho. violaceum</i>	2	2/2	0/0	0/0	0	0	0	
<i>Tricho. tonsurans</i>	1	0/0	0/0	1/1	45	42	0	
<i>Epidermo. floccosum</i>	5	0/1	0/0	5/4	36 to 60	17 to 57	0	
<i>Candida tropicalis</i>	2	0/2	0/0	2/0	21 to 42	0	0	
<i>Candida albicans</i>	2	2/2	0/0	0/0	0	0	0	
<i>Candida stellatodea</i>	1	0/0	1/1	1/1	34	17	0	

K – Ketoconazole  
G – Griseofulvin.

All the S isolates of *Epiderm. floccosum* tested were sensitive to Ketoconazole, but only four were sensitive to Griseofulvin.

All the isolates of *Tricho. mentagrophytes* were resistant to both the drugs. In two cases, the growth was present even in the punched holes containing the drugs (Fig.I).

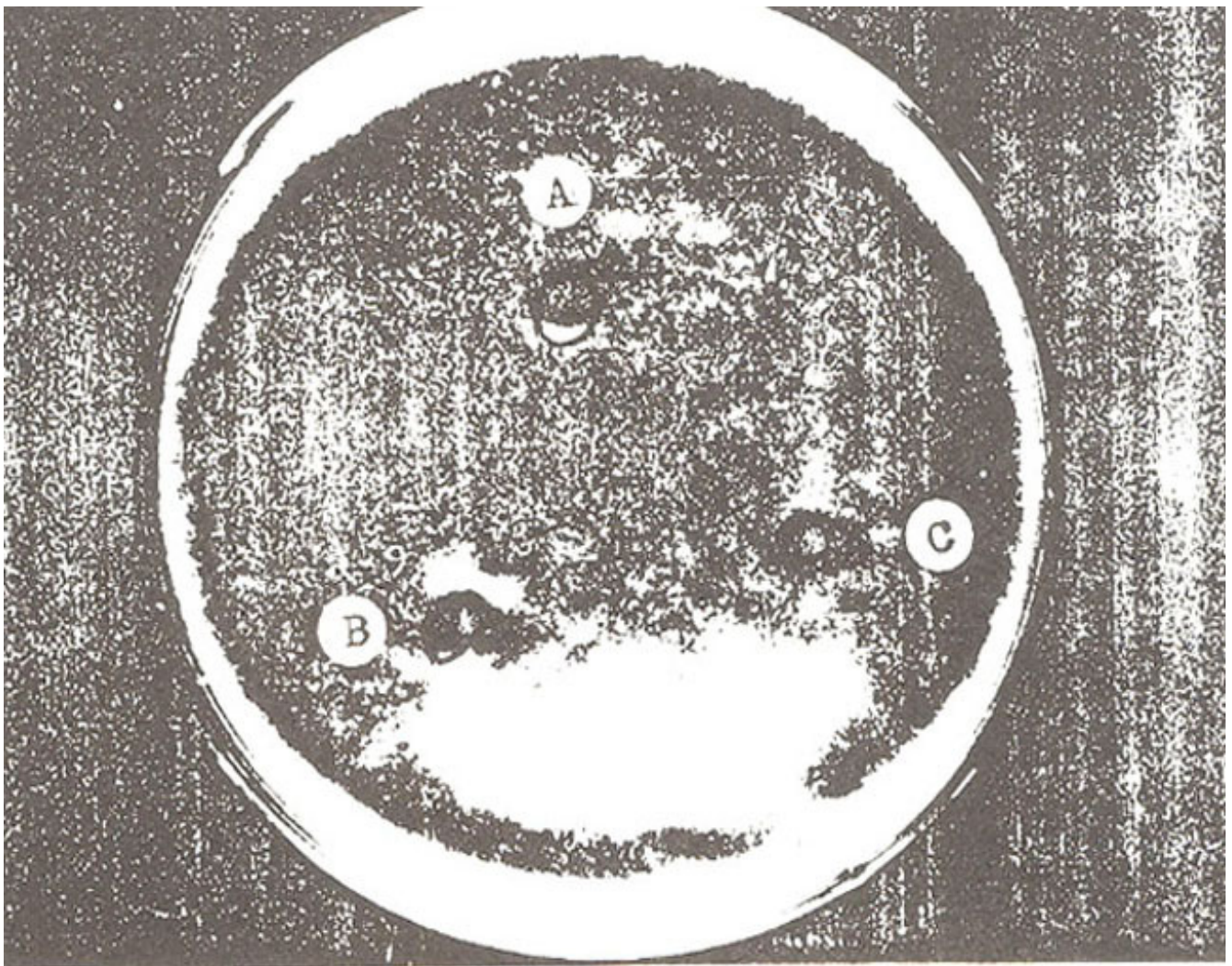


Fig. 1. *Tricho. mentagrophytes*: it is resistant to both the drugs.

- A. Control
- B. Ketoconazole
- C. Griseofulvin.

Of the 10 isolates of *Tricho. Schoenleini*, 9 were sensitive to Ketoconazole and 4 to Griseofulvin (Fig. 2).

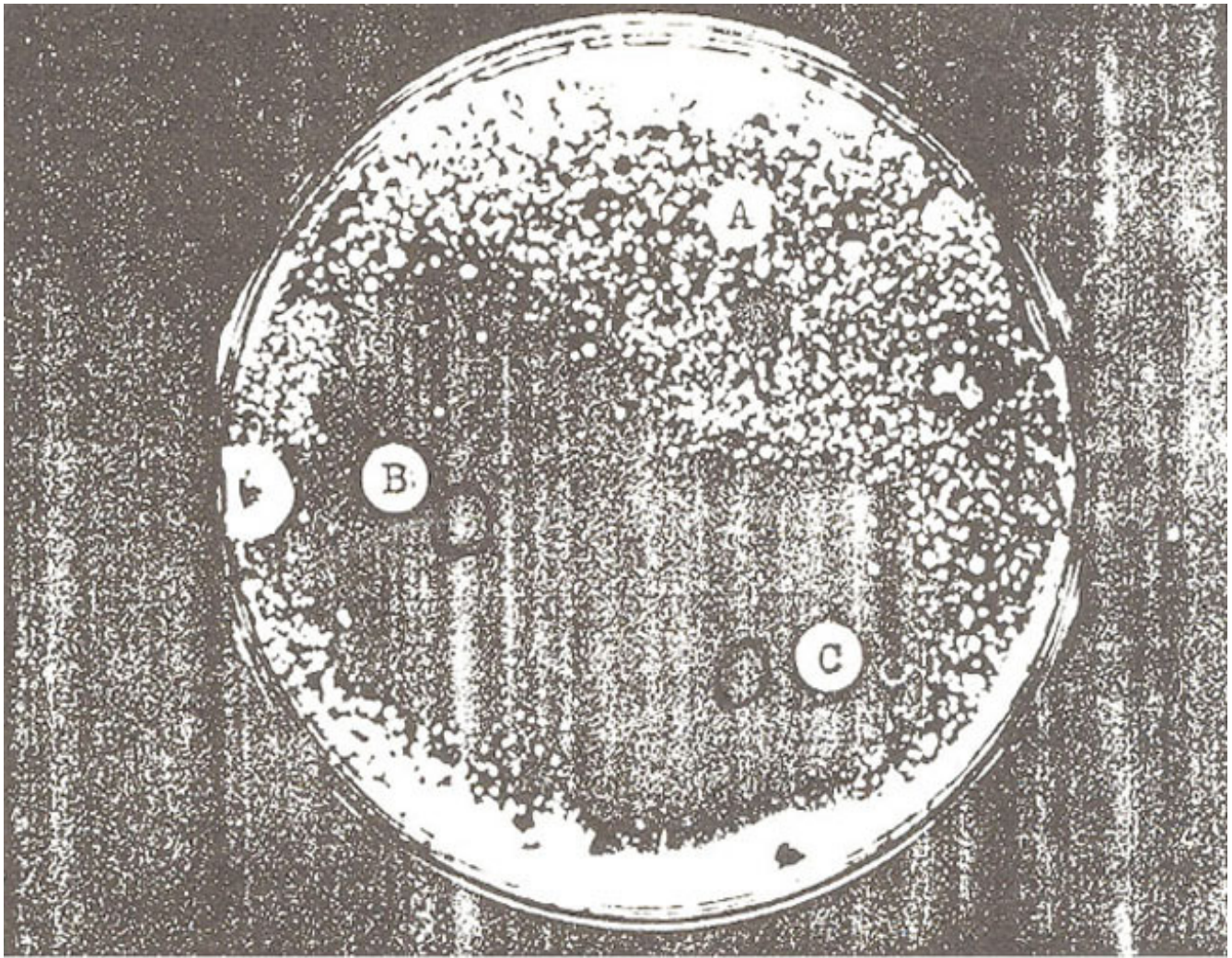


Fig. 2. *Tricho. schoenleini*: it is sensitive to both the drugs.

- A. Control
- B. Ketoconazole
- C. Grizeofulvin.

All the 8 isolates of *Mic. ferrugineum* were resistant to both the drugs, except two which were intermediately sensitive to Ketoconazole (Fig. 3).

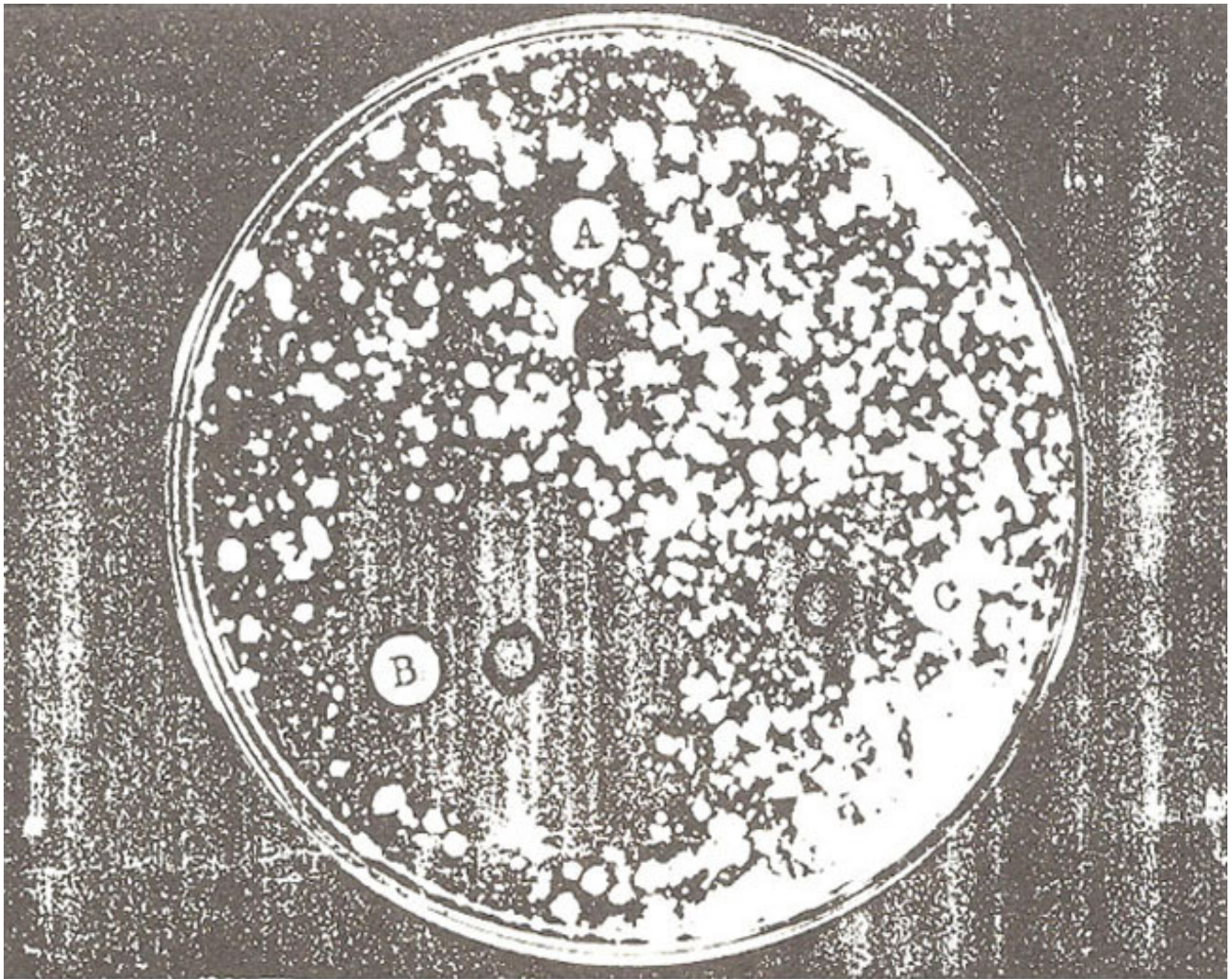


Fig. 3. *Mic. ferrugineum*: it is sensitive to Ketoconazole but resistant to Griseofulvin.

- A. Control
- B. Ketoconazole
- C. Griseofulvin.

Both the isolates of *Trich. violaceum* were resistant to both the drugs.

The only isolate of *Trich. tonsurans* tested was sensitive to both the drugs.

There were 5 isolates of *Candida* spp. including 2 of *Candida tropicalis*, which were sensitive to Ketoconazole but resistant to Griseofulvin; one of *Candida stellatoidea*, which was sensitive to both the drugs ; and two of *Candida albicans*, which were resistant to both the drugs.

### Discussion

In this in vitro study it was found that most of the dermatophytes and *Candida* spp. isolated were sensitive to Ketoconazole. The results with Ketoconazole were superior to those with Griseofulvin, as greater number of isolates were sensitive to it.

Similar results have also been found in other studies, where not all the isolates of a particular strain were sensitive to any of these drugs, although the results with Ketoconazole have been better than with Griseofulvin.

In a study conducted by Heel et al. (1982); it was found that after a median treatment period of 5 weeks, remission had occurred in 28 percent of cases, marked improvement in 30 percent and moderate improvement in 28 percent. Results were significantly better in patients with dermatophyte infections of human type, than when fungi from animal sources were involved. A high proportion of patients, followed for upto 3 months after discontinuing therapy (46 of 64 patients) suffered a relapse of their dermatomycosis. This shows that dermatophytes had not been completely eradicated.

In Candidiasis also, the remission with Ketoconazole occurred in only 77 percent of patients, after oral therapy with a dose of 200 or 400 mg. daily.

In another double blind comparative study of Ketoconazole (200 mg) and Griseofulvin (250 mg ultramicrosize) clinical remissions were induced in 61 percent and 39 percent of treated cases respectively (Hay,1982), but there is no mention of relapse rate.

The above study confirms that Ketoconazole has a broad spectrum of antifungal activity against dermatophytes and *Candida* spp. although not all the isolates were sensitive to it yet it was superior in its activity to Griseofulvin.

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