# Antifungal Susceptibility Patterns of Dermatophytes Clinical Isolates from Dermatophytosis Patients before and After Therapy

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## ABSTRACT

*The determination of fungus in vitro antifungal susceptibility has been reported to be important for the ability to eradicate pathogenic dermatophytes. This work aims to assess the in vitro activity of fluconazole, ketoconazole and itraconazole against dermatophytes from dermatophytosis patients before and after oral antifungal therapy with itraconazole. The study was conducted on 80 patients with dermatomycosis attending the Deramtology Outpatient Clinic of Zagazig University Hospitals. The patients were clinically diagnosed and mycologically confirmed as having tinea capitis (42), tinea corporis (18), tinea pedis (12), tinea ungium (5) and tinea cruris (3). All patients received a course of pulse itraconazole therapy. The clinical specimens were collected from all patients before and after 3 months of itraconazole oral therapy. Identification of dermatophytes to the species level was performed by colony morphology, microscopy and biochemical and physiological tests. All dermatophytes isolates were subjected to antifungal susceptibility testing by E-test method. In this work, the most frequently isolated species was T. rubrum, comprising 25 (31.25%) isolates, followed by T. mentagrophytes (21 isolates, 26.25%), T. violacium (17 isolates, 21.25%), M. canis (7 isolates, 8.75%), T. schoenlinii (6 isolates, 7.5%) and M. audouinii (4 isolates, 5%). The most active agent against all dermatophytes species was itraconazole with an MIC range of 0.094 – 12 µg/ml., MIC50 values of 0.125-0.5 µg/ml and MIC90 values of 0.25-8 µg/ml., followed by ketoconazole (MIC range of 0.0.64-24 µg/ml., MIC50 values of 0.38-1 µg/ml. and MIC90 values of 2-8 µg/ml). The least active agent was fluconazole (MIC50 of 64-≥256 µg/ml. and MIC90 of 128-≥256 µg/ml). MIC values higher than MIC90 were observed for the azole drugs when testing isolates obtained post-treatment from four tinea unguium patients. In conclusion, our data showed that itraconazole was the most active azole against all dermatophytes isolates. Furthermore, the increase in MIC values for azole drugs found for some of our isolates after therapy might raise the possibility of increased antifungal resistance. Further studies on larger samples of dermatophytes are recommended to correlate the MIC values with the clinical outcome for each isolate-drug combination to allow clinician adapting different therapeutic options. In addition, these studies could be beneficial for investigation of development of in vitro resistance in dermatophytes species, and for management of cases clinically unresponsive to treatment.*

## INTRODUCTION

Dermatophytes are a group of closely related fungal species that have the capacity to invade keratinized tissue of human and other vertebrates and produce dermatophytosis. Infections caused by these fungi are among the most prevalent cutaneous infections globally and the recent increase in the number of patients with immunocompromised states, such as AIDS, diabetes mellitus, cancer and organ transplantation has given these infections more prominence[(1-2)]

The treatment of dermatophytosis is based on the use of topical and systemic antifungal agents. While topical application of an antifungal is usually sufficient to eradicate the organism and to cure the majority of these infections, the most severe and chronic dermatophytosis, which includes some tinea unguium, scalp ringworm and skin lesions with folliculitis, often requires the administration of systemic treatment [(3)]

Although an increasing number of antimycotics has become available for the treatment of dermatophytosis, there are reports suggesting recalcitrance to therapy or possibly even resistance of dermatophytes to antimicrobial agents[(4)]. In order to predict the ability of a given antimycotic agent to eradicate dermatophytes and help managing patients, determination of the in vitro antifungal susceptibility of dermatophytes would be helpful in understanding a failed or successful treatment[(5)]. However, studies on the correlation of minimum inhibitory concentration (MIC) values to the clinical outcome are rare. Moreover, dermatophytes have not been included in the in vitro method proposed by the Clinical Laboratory Standards Institute (CLSI) for testing filamentous fungi[(6)].

This work aims to assess the in vitro activity of fluconazole, ketoconazole and itraconazole against dermatophytes from dermatophytosis patients before and after oral antifungal therapy with itraconazole.

## PATIENTS, MATERIALS & METHODS

This study was conducted on 80 patients with dermatomycosis of skin, hair and nail attending the Deramtology Outpatient Clinic of Zagazig University Hospitals. Their age ranged from 12 to 56 years (21.7±14.5 SD). They were 48 females and 32 males. The patients were clinically diagnosed and mycologically confirmed as having tinea capitis (42 case), tinea corporis (18 cases), tinea pedis (12 cases), tinea ungium (5 cases) and tinea cruris (3 cases). All patients received a course of pulse itraconazole therapy. The dosage of itraconazole was approximately 5 mg/Kg daily to twice-daily for one week of each of the 3-months course (from 2 weeks to 3 months)[7].

**Clinical specimens**

The clinical specimens including cutaneous skin scales, hair and nail scrapings or eclipses were collected from all patients at two points: before and after 3 months of antifungal oral therapy with itraconazole. The specimens were subjected to:

- Direct microscopic examination using 10% KOH
- Culture on Sabouraud's dextrose agar with chloramphenicol and cyclohexamide (Oxoid, UK). The cultures were incubated at 28ºC and examined daily for 30 days.
- Identification of dermatophytes to the species level was performed by colony morphology, microscopy and physiological and biochemical tests including urease and pigment production tests[8]

**Antifungal susceptibility testing**

All dermatophytes isolates were subjected to antifungal susceptibility testing by E-test method according to the manufacturer's instructions using E-test strips (AB Biodisk, Slona, Sweden) for fluconazole, ketoconazole and itraconazole.

For each isolate, a suspension of mycelia from a 7-day culture was prepared in saline to a concentration of $10^6$cells/ml. The suspensions were streaked into Sabouraud's glucose agar with the aid of moistened swabs. A strip of E-test was then carefully placed on the center of each plate and incubated at 28ºC for reading at 96 hours. The MIC values were the drug concentrations at which the border of the elliptical inhibition zone intersected the scale on the antifungal strip. The MIC50 values were the MIC values which inhibited 50% of all isolates while MIC90 inhibited 90% of all isolates. Two quality control strains were included: Candida parapsilosis (ATCC 22019) and Trichophyton rubrum (ATCC 40051)[9].

## RESULTS

In this work a total of 80 dermatophytes strains were isolated from all patients. The most frequently isolated species was *T. rubrum*, comprising 25 (31.25%) isolates, followed by *T. mentagrophytes* (21 isolates, 26.25%), *T. violacium* (17 isolates, 21.25%), *M. canis* (7 isolates, 8.75%), *T. schoenlinii* (6 isolates, 7.5%) and *M. audouinii* (4 isolates, 5%) as presented in table (1).

**Table (1):** The number and percentages of dermatophytes species according to the type of tinea

| Type of tinea (No.) | Dermatophytes species No. (%) | | | | | |
|---|---|---|---|---|---|---|
| | ***T. rubrum*** | ***T. mentagrophytes*** | ***T. violaceum*** | ***T. schoenlinii*** | ***M. canis*** | ***M. audouinii*** |
| Tinea capitis (42) | 0 (0) | 15 (35.7) | 12 (28.6) | 6 (14.3) | 5 (11.9) | 4 (9.5) |
| Tinea corporis (18) | 7 (38.7) | 5 (27.8) | 4 (22.2) | 0 (0) | 2 (11.1) | 0 (0) |
| Tinea pedis (12) | 10 (83.4) | 1 (8.3) | 1 (8.3) | 0 (0) | 0 (0) | 0 (0) |
| Tinea ungium (5) | 5 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Tinea cruris (3) | 3 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Total (80) | 25 (31.25) | 21 (26.25) | 17 (21.25) | 6 (7.5) | 7 (8.75) | 4 (5) |

The MIC values of fluconazole, ketoconazole and itraconazole against the 80 dermatophytes clinical isolates are summarized in table (2). The most active agent against all dermatophytes species was itraconazole with an MIC range of 0.094-12 µg/ml., MIC50 values of 0.125-0.5 µg/ml and MIC90 values of 0.25-8 µg/ml., followed by ketoconazole with MIC range of 0.064-24 µg/ml., MIC50 values of 0.38-1 µg/ml. and MIC90 values of 2-8 µg/ml. The least active agent was fluconazole with MIC50 of 64-≥256 µg/ml. and MIC90 of 128-≥256 µg/ml.

**Table (2):** The MICs of the three antifungals against 80 dermatophytes clinical isolates.

| Dermatophytes species | Antifungal agents (µg/ml.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fluconazole | | | Ketokonazole | | | Itraconazole | | |
| | MIC range | MIC50 | MIC90 | MIC range | MIC50 | MIC90 | MIC range | MIC50 | MIC90 |
| *T. rubrum* | 128 - ≥256 | 128 | 256 | 0.064 – 24 | 0.38 | 6 | 0.094 – 8 | 0.25 | 2 |
| *T. mentagrophytes* | 128 - ≥256 | ≥256 | ≥256 | 0.064 – 12 | 0.5 | 8 | 0.094 – 12 | 0.5 | 8 |
| *T. violaceum* | 64 – 256 | 64 | 128 | 0.125 – 4 | 1 | 2 | 0.094 – 2 | 0.125 | 0.25 |
| *T. schoenlinii* | 32 – 128 | N.C. | N.C. | 0.125 – 2 | N.C. | N.C. | 0.094 – 2 | N.C. | N.C. |
| *M. canis* | 128 - ≥256 | N.C. | N.C. | 0.064 – 2 | N.C. | N.C. | 0.094 – 4 | N.C. | N.C. |
| *M. audouinii* | 64 – 256 | N.C. | N.C. | 0.25 – 2 | N.C. | N.C. | 0.094 – 2 | N.C. | N.C. |

***N.C.:*** *Values not calculated for species with less than 10 isolates.*

After 3-month of antifungal oral therapy, 12 (15%) of 80 patients were not clinically cured, including 7 tinea pedis and 5 tinea ungium patients and mycological culture showed *T. rubrum* isolates. Table (3) shows the antifungal susceptibility data for these isolates from patients before and after therapy. Eight isolates showed the same or lower MIC values for the second isolates obtained from patients after therapy. Whereas, MIC values higher than MIC90 were observed for the azole drugs when testing isolates obtained post-treatment from four tinea unguium patients. MIC values for ketoconazole and itraconazole for the second isolates were two to four times higher than the MIC of these drugs for the first isolate from two patients (ketoconazole MIC90 of 12 µg/ml versus 6 µg/ml and itaconazole MIC90 of 8 µg/ml versus 2 µg/ml) and twice as high as the fluconazole MIC90 for the second isolates from two patients (MIC90 of ≥256 µg/ml versus 128 µg/ml).

**Table (3):** Antifungal susceptibility data for 12 *T. rubrum* clinical isolates from patients before and after therapy.

| Type of tinea | Isolate No | MIC data (µg/ml) | | |
|---|---|---|---|---|
| | | Fluconazole | Ketoconazole | Itraconazole |
| **Tinea ungium** | First | 256 | 6 | 2 |
| | **Second** | 128 | **12** | **8** |
| Tinea pedis | First | 256 | 4 | 2 |
| | Second | 256 | 4 | 1 |
| Tinea pedis | First | 256 | 12 | 2 |
| | Second | 256 | 4 | 2 |
| Tinea pedis | First | >256 | 6 | 2 |
| | Second | 256 | 6 | 0.75 |
| **Tinea ungium** | First | 256 | 6 | 2 |
| | **Second** | 128 | **12** | **8** |
| **Tinea ungium** | First | 128 | 6 | 4 |
| | **Second** | **256** | 4 | 2 |
| **Tinea ungium** | First | 128 | 6 | 2 |
| | **Second** | **>256** | 6 | 1 |
| Tinea pedis | First | 256 | 6 | 2 |
| | Second | 256 | 4 | 0.5 |
| Tinea ungium | First | >256 | 24 | 2 |
| | Second | >256 | 8 | 1 |
| Tinea pedis | First | 256 | 6 | 2 |
| | Second | 128 | 2 | 2 |
| Tinea pedis | First | 256 | 6 | 2 |
| | Second | 128 | 2 | 1 |
| Tinea pedis | First | 256 | 6 | 2 |
| | Second | 128 | 4 | 2 |

## DISCUSSION

The distribution of the dermatophytosis and their etiological agents has unequal frequencies, with variations of their prevalence according to the countries and even the regions of the same country. In this study, *T. rubrum* was the most frequently isolated organism (31.25%), followed by *T. mentagrophytes* (26.25%) and *T. violaceum* (21.25%). These results are in agreement with many other local[10] and international studies[1]. However, *T. violaceum* in Libya, *E. floccosum* in Iraq and *M. canis* in Italy are the most common cause of dermatophytosis[11-13].

The determination of fungus in vitro antifungal susceptibility has been reported to be important for the ability to eradicate pathogenic dermatophytes[5]. In this study, itraconazole had the highest antifungal activity for all dermatophytes species with the lowest MIC ranges among the tested azole drugs, including MIC50 (0.125–0.5 µg/ml) and MIC90 (0.25–8 µg/ml). Similar results have been verified by other researchers[14-16]. These data can help to explain the promising results obtained for the treatment of dermatophytosis with this antifungal agent[17]. The highest MIC values in this study were for fluconazole (MIC50: 64-256 µg/ml and MIC90: 128-≥256 µg/ml). Fernandez-Torres et al[14] studying trichophyton rubrum and trichophyton mentagrophytes reported MIC values for fluconazole of ≥256 µg/ml. The same results were mentioned by Barros et al[15].

One of the most striking findings of our study was the influence of E-test on MICs compared to values in other studies utilizing either the broth macro or microdilution methods[17-19]. A prominent increase in MIC values with wide MIC ranges was observed in this work. Mendez et al[19] compared two agar-based methods, E-test and the disk diffusion method with the CLSI reference broth microdilution method for susceptibility testing of 46 dermatophytes strains. These investigators found that the level of agreement between the E-test and broth microdilution method was 45.6% for fluconazole and 19.5% for itraconazole. On the other hand, Ferrnandez-Torres et al[14] found that the agreement between the E-test and microdilution method was 80%, 77% and 27% for itraconazole, ketoconazole and fluconazole respectively. It is difficult to compare results due to variability in critical technical factors in different studies, including inoculums size, type of media, incubation temperature and time of reading, which may explain the different results in antifungal susceptibility testing obtained by various investigators and laboratories[14-16,18&19]. Therefore, validation of the clinical significance of these observations demands determination of MIC breakpoints for dermatophytes and in vitro-in vivo correlation studies.

It is interesting to mention that MIC values higher than MIC90 were observed for the azole drugs when testing isolates obtained post-treatment from four tinea unguium patients in this work. In accordance, Santos et al[20] found that the MIC values of ketoconazole and itraconazole for the second isolate from two onychomycosis patients were four times higher than the MIC90 of these drugs for the first isolate and the sequential isolates from each patient exhibited the same genotype representing a single *T. rubrum* strain. Whereas, in other two patients, four times higher MIC values of itraconazole and miconazole for the second isolates were recorded and the sequential isolates from each patient were genetically unrelated representing two different *T. rubrum* strains. Moreover, in a study of Gupta and Kohli[21], which involved MIC determination for antifungal agents against sequential dermatophytes isolates obtained from patients who fail antifungal oral therapy, an increase in MIC values, especially for ketoconazole, when testing isolates post-treatment was observed. These researchers proposed the hypothesis that there are possibilities of increased resistance developing in the dermatophyte causative agent and of strain selection during the treatment. From these data, the higher MIC values of tested azoles for the second isolates obtained post-treatment in this work might be related to emergence of genetically different strain because of strain selection during the treatment or a possibility of increased resistance developing in the same pathogenic strain.

In conclusion, our data showed that itraconazole was the most active azole against all dermatophytes isolates, followed by ketoconazole and fluconazole. Furthermore, the increase in MIC values for azole drugs found for some of our isolates after therapy might raise the possibility of increased antifungal resistance. Further studies on larger samples of dermatophytes are recommended to correlate the MIC values with the clinical outcome for each isolate-drug combination to allow clinician adapting different therapeutic options with a high probability of successful results. In addition, these studies could be beneficial for

investigation of development of in vitro resistance in dermatophytes species, and for management of cases clinically unresponsive to treatment.

## REFERENCES


1. **Ozkutuk A, Ergon C and Yulug N. (2007):** Species distribution and antifungal susceptibilities of dermatophytes during a one year period at a university hospital in Turkey. Mycoses; 50: 125-129.
2. **Porro AM, Yosioka CN, Kaminiski SK, de Palmeira A, Fishman O and Alchorne M. (1997):** Dessiminated dermatophytosis caused by microsporum gypseum in two patients with the acquired immunodeficiency syndrome. Mycopathologia; 137: 9-12.
3. **Del Palcio A, Garau M, Gonzalez-Escalada A and Calvo MT. (2000):** Trends in the treatment of dermatophytosis. In: Kushwaha RKS, Guarro J, editors. Biology of dermatophytes and other keratinophylic fungi. Bilbao, Spain: Revista Iberoamericana de mycologia; P: 148-158
4. **Gupta AK and Kohli Y (2003):** In vitro susceptibility testing ciclopirox, terbinafine, ketoconazole and itraconazole against dermatophytes and non dermatophytes, and in vitro evaluation of combination antifungal activity. Br J Dermatol.; 149: 296-305.
5. **Cetinkaya Z, Kiraz N, Karaca S, Kulac M, Ciftci IH, Aktepe OC, Altindis M, Kiyildi N and Piyade M. (2005):** Antifungal susceptibility of dermatophytic agents isolated from clinical specimens. Eur.J.Dermatol.; 15: 258-261.
6. **Clinical Laboratory Standards Institute; CLSI (2002):** Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A, Wayne Pa.
7. **Hung PH and Paller AS (2000):** Itraconazole pulse therapy for dermatophyte onychomycosis in children. Arch.Pediatr.Adolesc.Med.; 154: 614-618.
8. **Larone DH (1995):** Medically important fungi: A guide to identification, 3rd ed., Washington, DC: ASM Press.
9. **Dos Santos JI, Paula CR, Viani FC and Gambale W. (2001):** Susceptibility testing Trichophyton rubrum and Microsporum canis to three azole antifungals by E-test. J.Med.Mycol.; 11: 42-43.
10. **Girgis S, Zu El-Fakkar N, Badr H, Shaker O and Bassim H (2006):** Genotypic identification and antifungal susceptibility pattern of dermatophytes isolated from clinical specimens of dermatophytosis in Egyptian patients. Egy.Dermatol.Online J.; 2: 1-23.
11. **Ellabib MS, Khalifa Z and Kavanagh K. (2002):** Dermatophytes and other fungi associated with skin mycosis in Tripoli, Libya. Mycoses; 156: 101-104.
12. **Muhsin TM, al-Rubaiy KK and al-Duboon AH. (1999):** Characteristics of dermatophytosis in Basrah, Iraq. Mycoses; 42: 335-338.
13. **Mercantini R, Moretto D, Palamara G and Marsella R. (1995):** Epidemiology of dermatophytosis observed in Rome, Italy between 1985 and 1993. Mycoses; 38: 415-419.
14. **Fernandez-Torres B, Carrillo-Munoz A, Ortoneda M, Pujolz I, Pastor FJ and Guarro J (2003):** Interalaboratory evaluation of the E-test for antifungal susceptibility testing of dermatophytes. Med.Mycol.; 41: 125-130.
15. **Barros ME, Santos DD and Hamdan JS (2007):** Antifungal susceptibility testing of trichophyton rubrum by E-test. Arch.Dermatol.Res.; 299: 107-109.
16. **Jessup CJ, Warner J, Isham N, Hasan I and Ghannoum MA (2000):** Antifungal susceptibility testing of dermatophytes: Establishing a medium for inducing conidial growth and evaluation of susceptibility of clinical isolates. J.Clin.Microbiol.; 38: 341-344.
17. **Olafsson JH, Sigurgeirsson B and Baran R (2003):** Combination therapy for onychomycosis. Br.J.Dermatol.; 149: 11-13.
18. **Siqueira ER, Ferreira JC, Pedroso RS, Lavrador MS and Candido RC (2008):** Dermatophyte susceptibilities to antifungal azole agents tested in vitro by broth macro and microdilution methods. Rev.Inst.Med.Trop.S.Paulo; 50: 1-5.
19. **Mendez CC, Serrano MC, Valverde A, Peman MC, Almeida C and Martin-Mazuelos E (2008):** Comparison of E-test, disk diffusion and a modified CLSI broth microdilution (M 38-A) for in vitro testing of itraconazole, fluconazole and voriconazole against dermatophytes. Med.Mycol.; 46: 119-123.
20. **Santos DA, Araujo RA, Kohler LM, Machado-Pinto JM, Hamdan JS and**

**Cisalpino PS (2007):** Molecular typing and antifungal susceptibility of trichophyton rubrum isolates from patients with onychomycosis pre- and post-treatment. Int.J.Antimicrob. Agents; 29: 563-569.

**21. Gupta AK and Kohli Y (2003):** Evaluation of in vitro resistance in patients with onychomycosis who fail antifungal therapy. Dermatol.; 207: 375-380.

# أنماط الحساسية للمضادات الفطرية للمعزولات السريرية للفطور الجلدية المعزولة من المرضى قبل وبعد العلاج

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يعتبر تحديد حساسية الفطر لمضادات الفطريات أمرا ضروريا للقضاء على الفطور الجلدية الممرضة.

يهدف هذا البحث إلى تعيين نشاط الفلوكونازول والكيتوكونازول والإيتراكونازول ضد الفطور الجلدية الممرضة والمعزولة من المرضى قبل وبعد العلاج عن طريق الفم بإستخدام الإيتراكونازول.

أجريت الدراسة على ٨٠ مريض مصاب بالقوباء الحلقية وأكد التشخيص السريرى والمزرعة الفطرية على أنها ٤٢ حالة مصابة بسعفة الرأس و١٨ حالة سعفة الجسدية و١٢ حالة سعفة القدم و٥ حالات سعفة أظافر اليد و٣ حالات سعفة الثنيات ، وتلقى جميع المرضى نبض العلاج بالإيتراكونازول لمدة تراوحت من أسبوعين إلى ٣ أشهر. تم تجميع العينات من جميع المرضى قبل وبعد العلاج ، وتم التعرف على أنواع الفطور الجلدية بإستخدام الفحص الميكروسكوبى وشكل المستعمرات الفطرية والإختبارات الفسيولوجية والكيميائية، وتعرضت جميع المعزولات الفطرية لإختبار الحساسية لمضادات الفطور بإستخدام طريقة "إختبار إى" بإستعمال شرائط الفلوكونازول والكيتوكونازول والإيتراكونازول.

أسفرت نتائج هذا البحث إلى أن الإيتراكونازول هو أكثر أنواع الأزول نشاطا ويليه الكيتوكونازول ثم الفلوكونازول وهو الأقل نشاطا. وقد أظهرت بعض معزولات الفطور إرتفاعا كبيرا فى الحد الأدنى للتركيز المثبط لمضادات الفطور بعد فترة العلاج مما قد يطرح إمكانية زيادة مقاومة الفطور الجلدية للمضادات الفطرية ، ونوصى بأبحاث أخرى على عينات أكثر من الفطور الجلدية لدراسة نشاط مضادات الفطور ضد أنواع الفطور الجلدية المختلفة مما قد يسمح للأطباء بإستخدام خيارات علاجية مختلفة لتحقيق نتائج ناجحة ، كما أن هذه الدراسات قد تكون مفيدة فى التحقق من وجود مقاومة الفطور للمضادات الفطرية والتعامل مع الحالات التى لا تستجيب للعلاج.