In-vitro Activity of 10 Antifungal Agents against 320 Dermatophyte Strains Using Microdilution Method in Tehran

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Abstract

Dermatophyte fungi are the etiologic agents of skin infections commonly referred to as ringworm. These infections are not dangerous but as a chronic cutaneous infections they may be difficult to treat and can also cause physical discomfort for patients. They are considered important as a public health problem as well. No information is available regarding the efficacy of antifungal agents against dermatophytes in Tehran. Therefore, in this study we evaluated the efficacy of 10 systemic and topical antifungal medications using CLSI broth microdilution method (M38-A). The antifungal agents used included griseofulvin, terbinafine, itraconazole, ketoconazole, fluconazole, voriconazole, clotrimazole, ciclopirox olamine, amorolfine and naftifine. Fifteen different species of dermatophytes which were mostly clinical isolates were used as follows; T. mentagrophytes, T. rubrum, E. floccosum, M. canis, T. verrucosum, T. tonsurans, M. gypseum, T. violaceum, M. ferruginum, M. fulvum, T. schoenleinii, M. racemosum, T. erinacei, T. eriophyton and Arthroderma benhamiae. The mean number of fungi particles (conidia) inoculated was 1.25 ×10⁴ CFU/mL. Results were read after 7 days of incubation at 28 °C. According to the obtained results, itraconazole and terbinafine showed the lowest and fluconazole had the greatest MIC values for the most fungi tested. Based on the results, it is necessary to do more research and design a reliable standard method for determination of antifungal susceptibility to choose proper antibiotics with fewer side effects and decrease antifungal resistance and risk of treatment failure.

Introduction

Cutaneous mycoses are divided into two groups based on their etiologic agent, including dermatomycosis and dermatophytosis. Dermatomycosis are cutaneous mycoses caused by any fungi other than dermatophytes. Dermatophytes are a closely related group of fungi in 3 anamorphic genera of Trichophyton, Microsporum and Epidermophyton that originate from humans, different animals or soil. Except for a few systemic diseases and immunosuppressed patients (1-7), they usually invade only keratinized tissue, such as skin epidermis, nail plate and hair. Although there are approximately more than 40 different species in these three genera, most infections are due to Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum

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and *Microsporum canis*. A single species may be seen in several disease types.

Griseofulvin was the only approved systemic antidermatophytic agent, however, at present new antifungal drugs with fewer side effects is available. Terbinafine (allylamines), ketoconazole (imidazole group), itraconazole, and fluconazole (triazoles group) are mostly used orally, and clotrimazole (imidazole), ciclopirox olamine (substituted pyridine), amorolfine (morpholin) and naftifine (allylamines) are usually applied topically for the treatment of these infections. Treatment failure occurs in 25-40% of patients with onychomycosis and 6.5% of those with tinea corporis and tinea cruris (8). Although, role of drug resistance in treatment failure is not clearly known, *in-vitro* susceptibility testing could help the clinicians to select the proper antifungal agent. There is no standard method available for susceptibility testing of dermatophytes. Determination of MIC (Minimal Inhibitory Concentration) by CLSI dilution method (M38-A) has been suggested in several studies (3, 9-18). There are more than 33 articles published on epidemiology, clinical aspects and etiology of dermatophytosis from Iran. However, only few articles studing antifungal susceptibility with the different method for these fungi (19-24). According to CLSI protocol M38-A, completely synthetic medium RPMI1640 (without sodium bicarbonate and L-glutamine at pH 7.0) supplemented with 0.165M morpholinepropanesuphonic acid (MOPS) is suitable for broth dilution based antifungal susceptibility determination for filamentous fungi (15). However, there are differences in time and incubation periods, form and numbers of inoculated fungi and MIC breakpoint determination and culture media used for different experiments.

Correlations of MICs with clinical outcomes have not been determined (10). It seems that comparing relying on the crude results is difficult, thus biostatic methods offered calculation of some parameters, like geometric mean (GM). Definitely, the geometric mean is the nth root of products of the value in a series of n-values. The geometric mean is more useful and representative than the arithmetic mean when describing a series of reciprocal or fractional values. The geometric mean can be used only for positive values (25).

The purpose of this study was to determine the susceptibility of 10 oral and topical antifungal agents including fluconazole, itraconazole, ketoconazole, voriconazole, clotrimazole, terbinafine, naftifine, amorolfine, ciclopirox olamine and griseofulvin against clinical dermatophytes isolated from patients in Tehran, Iran.

**Experimental**

**Dermatophyte strain**

A total of 320 dermatophyte strains belonging to 15 species were recognized using conventional methods such as slide culture, growth in CMA with 1% glucose, sabouraud dextrose agar with 3% NaCl, hair perforation, growth at 37°C, urease activity, and Trichophyton agar. *T. eriotrephon*, and *Arthroderma benhamiae* were diagnosed based on sequencing. Except *M. fulvum*, all other strains were clinical isolates from different laboratories in Tehran and included *T. rubrum* (n=89), *T. mentagrophytes* (n = 136), *E. floccosum* (n = 46), *T. verrucosum* (n = 11), *T. violaceum* (n = 2), *T. tonsurans* (n = 8), *M. canis* (n = 16), *M. gypseum* (n = 4), *M. ferruginum* (n = 2), *Arthroderma benhamiae* (n = 1), *M. racemosum* (n = 1), *T. erinacei* (n = 1), *M. fulvum* (n = 1), *T. schoenleinii* (n = 1) and *T. eriotrephon* (n = 1). Four standard strains cultures of *T. rubrum* (ATCC MRL 666), *Aspergillus fumigatus* (ATCC304305), *Candida krusei* (ATCC 6258), and *Candida parapsilosis* (ATCC 22019) were also used for quality control of tests. Prior to testing, the fungi were sub-cultured on sabouraud CAF agar with actidione (Liofilchem, Italy) at 28°C for 7 to 15 days to ensure purity, and then on potato dextrose agar (PDA) (Liofilchem, Italy) for sporulation of the inoculum. Tests were performed using NCCLS(CLSI) broth micro dilution method (M38-A) (15), as coming below.

**Culture media**

All dermatophyte strains were on RPMI 1640 medium (Sigma – Aldrich Chemie, GmbH, Riedstr) with L-glutamine and without sodium bicarbonate buffered at pH 7.0 with 0.165
M morpholinepropanesulfonic acid (MOPS) (Sigma, Aldrich Chemie, GmbH, Riedstr).

**Antifungal agent dilution**

Crude powder of antifungal drugs, including fluconazole, ketoconazole, itraconazole, clotrimazole, ciclopirox olamine, terbinafine, griseofulvin (Sigma–Aldrich Chemical Company, GmbH, Riedstr), naftifin ehydrochloride (Toronto Research Chemical Inc. Canada), amorolfine hydrochloride (Tokyo Chemical Industry Co. Toshima, KITA –KU, Tokyo, Japan) and voriconazole (Pfizer SA Amboise Cedex, France), were prepared by dissolving the powders in their specific solvents. Fluconazole dissolved in distilled water, while other drugs dissolved in 100% dimethyl sulfoxide (DMSO, Sigma–Aldrich).

Final concentrations of antifungal drugs ranged between 0.0625 and 256 µg/mL for fluconazole, 0.0312 and 32 µg/mL for ketoconazole, clotrimazole, ciclopirox, and naftifine, 0.0078 and 16 µg/mL for voriconazole, 0.0078 and 8 µg/mL for itraconazole, 0.0156 and 16 µg/mL for terbinafine, and 0.0312 and 256 µg/mL for griseofulvin.

**Inoculum preparation**

Stock inoculum suspensions of the fungi were prepared from 7 to 10 day old cultures grown on PDA at 28°C. Mature colonies were covered with approximately 5 mL of sterile saline (0.85%) by scraping the surface with the tip of a sterile swab. The resulting mixture of conidia and hyphal fragments was withdrawn and transferred to sterile tubes. Heavy particles were allowed to sediment for 15 to 20 min at room temperature.

The upper suspension was transferred to another sterile tube and mixed with a vortex mixer for 15 sec. Before the turbidity of the supernatants was measured spectrophotometrically (Jenway Model 6305) at a wavelength of 530 nm, transmission was adjusted to 65 by ETO sterilized disposable cuvette. Each suspension was diluted 1:50 to 1:100 in RPMI 1640 to obtain twice the final test inoculum concentration. The inocula corresponding to the strains were quantified by plating 5 µL of a 1:100 dilution of the adjusted inoculum on PDA plates and spread in 4 directions by a sterile loop. The plate contents were incubated at 30°C and observed daily for the presence of fungal colonies. Colonies were counted, when the growth became visible, as number of CFU per milliliter.

**Test procedure**

All the tests were performed in sterile, flat-bottomed, 96-well micro plates (Orange Scientific, E.U.). Aliquots of 100 µL of the 2× drug dilutions were inoculated into the wells with a multichannel pipette. The micro plates were put in storage at -22°C until used.

For performing the susceptibility testing, 100 µL of the diluted inoculum suspensions was added to each well to bring the drug dilutions to the final test concentrations. Growth and sterility control wells were also prepared for each isolate tested. The micro plate contents were incubated at 28 °C, avoiding desiccation of the wells, and were read visually with the aid of an inverted reading mirror after 7 days of incubation. Every test was repeated at least twice.

**Reading results**

For fluconazole and griseofulvin, the MIC was defined as approximately 50% growth inhibition compared to the growth control well. For other antifungal agents, the MIC was defined as the lowest concentration showing 100% growth inhibition.

**Data analysis**

Geometric mean, MIC range, MIC50, MIC80 and MIC90 were obtained for all the isolates tested. MIC value of antifungal drugs for different species were compared by repeated measure and one-way ANOVA and post-hoc Scheffe tests using SPSS version 16 software. P-values < 0.05 were considered statistically significant.

**Results and Discussion**

A total of 320 dermatophytes, mostly clinically isolated from patients, were tested using modified M38 –A CLSI micro dilution method. T. mentagrophytes, followed by T. rubrum and E. floccosum, were the most common dermatophytes isolated from patients in Tehran.

In-vitro activities of 10 antifungal agents that
| Species (number of strain tested) | Concentration (µg/mL) |  |  |  |  |  |  |  |  |  |  |
|----------------------------------|-----------------------|----|----|----|----|----|----|----|----|----|----|
|                                 | Terb                 | Gri | Itra | keto | Flu | Vor | Clz | Ciclo | Amor | Naft |
| T. rubrum (n = 89)              | GM                    | 0.172 | 1.61 | 0.06 | 0.67 | 11.05 | 0.19 | 0.34 | 1.52 | 0.316 | 0.182 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 1 | 32 | 0.125 | 0.25 | 2 | 0.25 | 0.125 |
|                                 | MIC80                 | 8 | 128 | 0.5 | 4 | 128 | 1 | 1 | 8 | 2 | 0.5 |
|                                 | MIC90                 | 16 | 256 | 2 | 8 | 256 | 1 | 4 | 16 | 4 | 4 |
| Range                           | 0.0156-16 | 0.0312-256 | 0.0009-4 | 0.0312-32 | 0.00625-256 | 0.00078-8 | 0.0312-16 | 0.0312-32 | 0.0078-32 | 0.0625-16 |
| GM                              | 0.142 | 2.66 | 0.045 | 0.94 | 18.82 | 0.28 | 0.247 | 2.26 | 0.389 | 0.147 |
| MIC50                           | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-16 | 0.0312-256 | 0.0009-4 | 0.0312-32 | 0.00625-256 | 0.00078-8 | 0.0312-16 | 0.0312-32 | 0.0078-32 | 0.0625-16 |
| T. mentagrophytes (n = 136)     | GM                    | 0.142 | 2.66 | 0.045 | 0.94 | 18.82 | 0.28 | 0.247 | 2.26 | 0.389 | 0.147 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-16 | 0.0312-256 | 0.0009-4 | 0.0312-32 | 0.00625-256 | 0.00078-8 | 0.0312-16 | 0.0312-32 | 0.0078-32 | 0.0625-16 |
| E. floccosum (n = 46)           | GM                    | 0.093 | 2.31 | 0.035 | 0.43 | 10.44 | 0.174 | 0.33 | 1.81 | 0.25 | 0.33 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-16 | 0.0312-256 | 0.0009-4 | 0.0312-32 | 0.00625-256 | 0.00078-8 | 0.0312-16 | 0.0312-32 | 0.0078-32 | 0.0625-32 |
| T. verrucosum (n = 11)          | GM                    | 0.062 | 1.99 | 0.0186 | 0.77 | 16 | 0.108 | 0.266 | 6.62 | 0.87 | 2.56 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-16 | 0.0312-256 | 0.0009-4 | 0.0312-32 | 0.00625-256 | 0.00078-8 | 0.0312-16 | 0.0312-32 | 0.0078-32 | 0.0625-32 |
| T. tonsurans (n = 8)            | GM                    | 0.088 | 24.24 | 0.147 | 0.79 | 13.92 | 0.39 | 0.297 | 3.17 | 0.87 | 0.31 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-8 | 0.0312-256 | 0.0009-1 | 0.0312-8 | 0.0625-256 | 0.0156-8 | 0.0312-2 | 0.0312-32 | 0.0078-2 | 0.0625-8 | 0.0312-16 |
| M. canis (n = 16)               | GM                    | 0.046 | 128 | 0.375 | 1 | 32 | 0.125 | 0.375 | 9 | 0.75 | 4.5 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-8 | 0.0312-256 | 0.0009-1 | 0.0312-8 | 0.0625-256 | 0.0156-8 | 0.0312-2 | 0.0312-32 | 0.0078-2 | 0.0625-8 | 0.0312-16 |
| M. gypseum (n = 4)              | GM                    | 0.046 | 2.82 | 0.051 | 3.36 | 45.25 | 0.353 | 0.176 | 16 | 0.297 | 0.125 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-8 | 0.0312-256 | 0.0009-2 | 0.0625-256 | 0.0156-8 | 0.0625-4 | 0.0625-2 | 0.0312-32 | 0.0078-2 | 0.0625-8 | 0.0625-0.25 |
| M. ferruginum (n = 2)           | GM                    | 0.044 | 0.16 | 0.08 | 0.31 | 11.98 | 0.164 | 0.20 | 1.64 | 0.183 | 0.2 |
|                                 | MIC50                 | 0.0312 | 2 | 0.0625 | 2 | 64 | 0.5 | 0.125 | 2 | 0.25 | 0.25 |
| Range                           | 0.0156-8 | 0.0312-256 | 0.0009-0.5 | 0.0625-4 | 0.0625-256 | 0.0156-8 | 0.0625-2 | 0.0312-32 | 0.0078-16 | 0.5-8 |
| M. racemosum (n = 1)            | GM                    | 0.046 | 2.82 | 0.051 | 3.36 | 45.25 | 0.353 | 0.176 | 16 | 0.297 | 0.125 |
|                                 | MIC90                 | 8 | 256 | 2 | 8 | 256 | 2 | 4 | 16 | 8 | 4 |
| Range                           | 0.0156-8 | 0.0312-256 | 0.0009-2 | 0.0625-256 | 0.0156-8 | 0.0625-4 | 0.0625-2 | 0.0312-32 | 0.0078-2 | 0.0625-8 | 0.0625-0.25 |
| M. fulvum (n = 1)               | GM                    | 0.0625 | 0.5 | 0.5 | 2 | 0.0625 | 0.3012 | 0.0625 | 0.25 | 0.25 | 0.25 |
|                                 | MIC50                 | 0.0625 | 0.5 | 0.5 | 2 | 0.0625 | 0.3012 | 0.0625 | 0.25 | 0.25 | 0.25 |

Table 1. In-vitro susceptibility of 10 antifungal drugs against 15 species of dermatophytes.
In-vitro Activity of 10 Antifungal Agents against 320 Dermatophyte Strains

potentially can be used either orally or topically, following micro dilution and 7 day incubation at 28 °C are reported in Table 1. Geometric mean MICs, MIC range, MIC 50, MIC 80 and MIC 90 were obtained for all the isolates tested.

Geometric mean (GM) of itraconazole and terbinafine was lowest for *T. rubrum, T. mentagrophytes* and *E. floccosum* indicating that these drugs are most potent in in-vitro studies.

Mean MICs of antifungal drugs did not show statistically significant differences between different species (p > 0.05). Voriconazole was more effective than ketoconazole, however fluconazole and griseofulvin had the lowest MIC against these 3 dermatophytes. Amongst topical antifungal agent, clotrimazole showed lowest MIC value followed by amorolfine, naftifine and ciclopirox olamine.

Itraconazole and terbinafine showed the lowest GM MIC for *M. canis*. Griseofulvin had GM MIC lower than voriconazole, followed by ketoconazole and fluconazole against this microorganism. After fluconazole, griseofulvin had the highest GM MIC for most other dermatophytes. Between 4 topical antifungal agents, clotrimazole had the lowest MIC and GM MIC. Amorolfinewas the second antifungal agent against *M. canis*, and naftifine and ciclopirox olamine were rated third and fourth respectively.

Although terbinafine had the greatest in-vitro activity and lowest GM MIC against *T. tonsurans, M. gypseum, Arthroderma benhamiae*, voriconazole had the lowest MIC against *T. violaceum*. A surprising result was that itraconazole was the most potent antifungal agent against most of the microorganisms tested in this study.

These tests were performed under variable conditions in terms of type of culture media for sporulation, incubation temperature, number and type of inoculated fungi and method, reading and interpreting the result by different researchers. Thus it would be difficult to compare the results.

The culture media used for sporulation in this study was the PDA media which was in according with many other studies like Li et al., Santos et al., Sarifakioglu et al., Galuppi et al., Ghannoum et al., Carrillo et al., Fernandez Torres et al., and Esteban et al. studies (8, 10, 12, 14, 16, 26-29).

A 7-10-day incubation time was allocated for sporulation of the majority of dermatophytes, like *T. mentagrophytes* and *T. rubrum*. Longer periods up to 6 weeks were required for other fungi, especially *T. schoenleini and T. violaceum* until the colony morphology changed to a white fluffy appearance. The incubation time used in this study was similar to the one used by Barroset al. (30), Santos et al. (27), and Fernandez Torrez et al. (12, 14).

For inoculation of fungal suspension into

### Table 1. (Continued)

| Strain                  | MIC  (n = 1) | GM  (n = 1) | MIC50 | MIC80 | MIC90 | Range |
|-------------------------|-------------|-------------|-------|-------|-------|-------|
| *T. schoenleinii*       | 0.0156      | 0.131       | 0.0312| 0.25  | 16    | 0.0156-16 |
| *E. terrirothoph*       | 0.0156      | 0.125       | 4     | 0.25  | 1     | 0.0156-16 |
| *T. eriotrephon*        | 0.0625      | 1.96        | 0.0625| 0.25  | 0.25  | 0.0625-0.0625 |
| *Arthroderma benhamiae*| 0.0156      | 1.96        | 0.0625| 0.25  | 0.25  | 0.0156-0.0625 |
| *T. schoenleinii*       | 0.0156      | 0.25        | 16    | 1     | 8     | 0.0156-16 |
| *T. eriotrephon*        | 0.0312      | 0.0625      | 1     | 0.25  | 2     | 0.0312-0.0625 |
| *T. eriotrephon*        | 0.0625      | 0.0312      | 2     | 0.25  | 2     | 0.0625-0.0312 |
| *Arthroderma benhamiae* | 0.0156      | 0.25        | 16    | 1     | 8     | 0.0156-16 |
| *T. schoenleinii*       | 0.0156      | 0.125       | 4     | 0.25  | 2     | 0.0156-0.0625 |
| *T. eriotrephon*        | 0.0312      | 0.0625      | 1     | 0.25  | 2     | 0.0312-0.0625 |
| *T. eriotrephon*        | 0.0625      | 0.0312      | 2     | 0.25  | 2     | 0.0625-0.0312 |
| *Arthroderma benhamiae* | 0.0156      | 0.25        | 16    | 1     | 8     | 0.0156-16 |
| *T. schoenleinii*       | 0.0156      | 0.0625      | 0.5   | 1     | 8     | 0.0156-0.0625 |
| *T. eriotrephon*        | 0.0312      | 0.0625      | 1     | 0.25  | 2     | 0.0312-0.0625 |
| *T. eriotrephon*        | 0.0625      | 0.0312      | 2     | 0.25  | 2     | 0.0625-0.0312 |
| *Arthroderma benhamiae* | 0.0156      | 0.25        | 16    | 1     | 8     | 0.0156-16 |

Abbreviations: terb, terbinafine; Gri, griseofulvin; Itra, itraconazole; Keto, ketoconazole; flu, fluconazole; Vor, voriconazole; Cli, clotrimazole; Ciclo, ciclopirox olamine; Amor, amorolfine; Naft, naftifine; GM, geometric mean; MIC50, MIC80, MIC90, MIC at which 50, 80 and 90% of fungi are inhibited respectively.
Fernandez-Torres had the lowest susceptibility, while in M. gypseum (GM=0.142) had the lowest susceptibility. In T. rubrum (GM=0.172) and T. mentagrophytes, the most sensitive dermatophytes, whereas similar (GM=0.04) and they were considered more sensitive than our study (11, 28). In this study, the GMs of dermatophytes in accordance with the instructions given by Favre et al., Esteban et al., and Fernandez-Torres et al. (12, 29).

The results of micro dilution tests for most strains read after 7 days at 28 °C, when adequate growth was observed in the control well with significant opacity. The 7 day time period has also been mentioned in Santos et al. (27), Gupta et al. (31), Fernandez-Torres (14), and Barros (30) studies. This time period was shorter (4 days at 35°C) in Ghannoum et al. (10), and Mukherjee (32) studies. This difference might be explained by the different temperatures used. Galuppi et al. (28) reported a longer period of 14 days and incubation at 30°C. This difference in the required incubation time may be due to the different volumes of fungi inoculated into the micro plates.

Antibiogram results for 320 dermatophytes showed that the highest susceptibility of dermatophytes was to itraconazole and the lowest to fluconazole. Our findings about susceptibility of dermatophytes to fluconazole is compatible with Santos et al. (27), Favre et al. (11), Barros et al. (30) and Sarifakioglu et al. (8) studies. Korting et al. (33) suggested that high values of MIC for fluconazole may be due to technical problems, such as interference with some ingredients of the culture media or insolvability at high concentrations.

However, geometric means MIC (GM) obtained in this study showed that the results of terbinafine for all species of dermatophytes were significantly greater than results of Gupta et al. study (31), but were in agreement with Munoz et al., Esteban et al. and Fernandez-Torres et al. (12, 16, 29) findings. The GMs of dermatophytes in Favre et al., and Galuppi et al. study were greater than our study (11, 28). In this study, the GMs of M. gypseum, M. canis and T. violaceum were similar (GM=0.04) and they were considered the most sensitive dermatophytes, whereas T. rubrum (GM=0.172) and T. mentagrophytes (GM=0.142) had the lowest susceptibility. In Esteban et al. (29) study, T. rubrum (GM=0.190) had the lowest susceptibility, while in Fernandez-Torres et al. study, T. rubrum and T. violaceum (GM=0.01) were the most sensitive dermatophytes (12). Considering the fact that laboratory methods were similar in these studies and also for determining the MIC endpoint for terbinafine is usually selected a well with 100% growth inhibition, it appears that the reported differences might be due to the differences between strains or different number of fungi tested.

In the present study, T. tonsurans (GM=24.24) showed the lowest and M. canis (GM=0.16) showed the highest susceptibility to griseofulvin. This is in complete agreement with Seebacher et al., opinion, and they suggest that griseofulvin is the best choice in the treatment Tinea capitis cases due to M. canis (18). T. mentagrophytes and E. floccosum have much lower susceptibility to griseofulvin than T. rubrum. Chadeganipour et al. (24) also reported higher MIC value for T. mentagrophytes than other tested dermatophytes. The GM reported by Galuppi et al. was also higher than our finding (GM=2.20) (28). However, Favre et al. reported an even smaller value (GM=0.37)(11). These differences are justifiable considering different readings/interpretations as 50%, 100% and 75% growth inhibition, respectively, compared to the growth control well of micro plate for determining MIC.

Itraconazole MIC results showed that GMMIC of T. rubrum in our study (GM=0.06) was much smaller than Gupta et al. (GM=0.143), Fernandez-Torres et al. (GM=0.09), Esteban et al. (GM=0.912), and Galuppi et al. (GM=0.96) findings. Gupta et al., and after that Fernandez-Torres et al. (31) were obtained the lowest GM for T. mentagrophytes. We also found the lowest GM for T. mentagrophytes. Esteban et al., followed by Galuppi et al, found the highest GM for this fungi (12, 28, 29, 31).

Susceptibility of T. rubrum with GM=0.67 to ketoconazole in this study was almost similar to Fernandez-Torres et al., (GM=0.65) and Galuppi et al., (GM=0.76) study, but was significantly higher than those obtained by Favre et al., (0.22), Fernandez et al. (0.14) and Gupta et al. (0.165) (11, 12, 14, 28, 31, 34).

In the present study, T. mentagrophytes after M. gypseum showed the lowest and E. floccosum had the highest in-vitro sensitivity to fluconazole.
The GM in our study was much greater than the GMs obtained in Favre et al. (11), (GM=6.3) and Carrillo et al. (16), (M=15.08) study. In Carrillo et al. (16) study, M. gypseum and T. mentagrophytes had the highest GM which means the lowest susceptibility. Kotring et al. (33) showed that the concentration of this drug, following consumption of 150 mg orally after 7 days, reaches to 7.1 μg/mL in stratum corneum. In addition, MIC > 64 μg/mL is attributed to resistance to this drug (35, 36). MIC mean values for half of the dermatophytes strains in our study is about 7 times greater than in-vivo condition, used for treatment of dermatophyte infections. In Foroumadi et al. (37) study, high MIC value were seen in cases of vulvovaginal candidiasis in Tehran treated with fluconazole, too.

In this study, we examined voriconazole antifungal susceptibility against dermatophytes. However, this drug is not currently being used due to its high cost. According to our study, T. schoenlinii, T. violaceum, T. verrucosum, M. canis, E. floccosum, T. rubrum, T. mentagrophytes, M. gypseum and T. tonsurans had, in an ascending order, the lowest to highest susceptibility. The GMs for all strains were greater than those obtained in Carrillo et al, Favre et al, and Fernandez - Torres et al. studies. However, Fernandez-Torres et al. and Carrillo et al, showed the highest susceptibility of E. floccosum to voriconazole (11, 12, 14, 16, 38).

Among topical antifungal agents that are usually used along with systemic antifungal medications, we selected ciclopirox olamine, clotrimazole, amorolfine and naftifine for in-vitro antifungal susceptibility. Amorolfine and naftifine are not available in Iran and therefore are not prescribed. In our study, M. gypseum (GM=0.176) and T. rubrum (GM=0.34) had the highest and lowest susceptibility to clotrimazole, respectively. Fernandez-Torres et al. (14) found similar results and showed that M. gypseum was the most sensitive dermatophyte to clotrimazole. Whereas, in Fernandez - Torres et al. (12) study, M. canis along with T. rubrum had the highest susceptibility. Esteban et al. also reported different findings and indicated that T. tonsurans and M. canis had the highest susceptibility (29). This difference may be attributed to unequal size of inoculums.

Antibiogram of ciclopirox olamine showed that T. violaceum had the highest susceptibility, followed by Trubrum, M. canis, M. gypseum, E. floccosum, T. mentagrophytes, T. tonsurans, M. ferruginum and T. verrucosum in a decreasing fashion.

In terms of quantity, MIC50 values in our study, except for T. tonsurans, were in agreement with Korting et al. (33) findings. The figure obtained in our study was in the range of 0.125-2 μg/mL, while this was 2 or 3 μg/mL in Korting et al. study and both these rates were greater than figures obtained by Gupta et al, Santos et al, and Favre et al. studies (11, 27, 31).

Susceptibility testing of dermatophytes to amorolfine has been previously performed by Li et al and Favre et al. (11, 26). The lowest values (MIC50=0.004, and MIC90=0.008 μg/mL) were reported by Favre et al, and these values were almost similar for 20 dermatophytes. Li et al reported MIC50 and MIC90=0.04 μg/mL for T. rubrum, MIC50=0.04 and MIC90=0.8 μg/mL for T. mentagrophytes and MIC50=0.02 and MIC90=0.04 μg/mL for E. floccosum. Our findings for MIC50 and MIC90 for all strains were greater than the mentioned rates. The lowest rate of MIC50 belonged to M. canis (MIC50=0.0625 μg/mL) and the highest was reported for T. verrucosum (MIC50=1 μg/mL). The lowest MIC90 belonged to T. tonsurans (MIC90=2 μg/mL) and the highest to M. canis (MIC90=16 μg/mL). Thus, M. canis showed the widest MIC range.

Naftifine is a topical antifungal agent chemically belonging to the family of allylamines. For naftifine, the lowest value of MIC50 (MIC50=0.0625 μg/mL) belonged to T. violaceum and the highest to T. tonsurans (MIC50=4.5 μg/mL). MIC50 values for M. gypseum, E. floccosum, T. mentagrophytes and T. rubrum were exactly the same, but the MIC90 values were different from each other and the highest MIC90 belonged to E. floccosum (MIC90=22.4 μg/mL), followed by T. tonsurans (MIC90=16 μg/mL).

In conclusion, the present study reveals that 2 out of 10 antifungal agents used in this study had high MIC value against dermatophytes. The clinical significance of testing this group of fungi
remains uncertain, and breakthroughs with proven relevance have yet to be identified and approved by regulatory agency. Based on our results, griseofulvin and fluconazole for dermatophyte infection should be used with a greater caution. Controversial findings in our study and others point the necessity of establishing a standard method for antibiogram of dermatophytes to facilitate the selection of drug similar to what is routinely performed for yeasts and bacteria.

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References

(1) Erbagci Z. Deep dermatophytoes in association with atopy an diabet melitus majocchi’ s granuloma tricophytic dermatophytic pseudomycetoma. Mycopathologia. (2002) 154: 163- 9.

(2) Berg JC, Hamacher KL and Roberts GD. Pseudomycetoma caused by Microsporum canis in immunosuppressed patient: a case report and review literature. J. Cutan Pathol. (2007) 34: 431- 4

(3) Bueno JG,Martinez C, Zapata B, Sanclemente G, Gallego M and Mesa AC. In-vitro activity of fluconazole, itraconazole, voriconazole and terbinafen against fungi causing onychomycosis. Clinical and Experimental Dermatology. (2010) 35: 658-663.

(4) Ali SS, Zafar A., Muzaffar S, Aslam F and Khan JA. Bilateral breast enlargement due to Trichophyton rubrum. Breast. (2006) 15: 263-5.

(5) Gong JQ, Liu XQ, Zeng XS and Li XF. Deep dermatophytosis caused by Trichophyton rubrum : report of two cases. Mycoses (2007) 50: 102-8.

(6) Lillis JV, Dawson ES, Chang R and White CR. Dissemminated dermal Trichophyton rubrum infection - an expression of dermatophyte dimorphism? J. Cutan. Pathol. (2010) 37: 1168-9.

(7) Sarifakiouglu E, Seckin D, Demirbilek M and Can F. In-vitro antifungal susceptibility pattern of dermatophyte strains causing tinea unguium. Clinical and Experimental Dermatology. (2007) 32: 675-679.

(8) Nweze EI, Ogbonna CC and Okafor JL. In-vitro susceptibility testing of dermatophytes isolated from pediatric cases in Nigeria against five antifungals. Rev . Inst . Trop . S. Paulo,(2007) 49: 293-295

(9) Ghannoum MA, Chatuverdi V, Espinel - Ingoff A , Pfaller MA , Rinaldi MG, Lee - Yang W and Warnock DW. Intra -and interl aboratory study of a method for testing the antifungal susceptibilities of dermatophytes. J. Clinical Microbiology (2004) 42: 2977-2979.

(10) Favre B, Hofbauer B, Hildering KS and Ryder NS. Comparison of in-vitro activities of 17 antifungal drug against a panel of 20 dermatophytes by using a microdilution assay. J. Clinical Microbiolog. (2003) 41: 4817-4819.

(11) Fernandez-Torres B, Carrillo - Munos AJ, Martin E, Del Palacio A, Moore MK, Valverede A , . Serranno M and Guarro J. In-vitro activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrobial Agents and Chemotherapy. (2001) 45: 2524-2528.

(12) Perea S , Fothergill AW, Sutton DA and Rinaldi MG. Comparison of in-vitro activities of voriconazole and five established antifungal agents against different species of dermatophytes using a broth microdilution method. J. Clinical Microbiology (2001) 39: 385-388.

(13) Fernandez - Torres B, Inza I and Guarro J. In-vitro activities of the new antifungal drug Eberconazole and three other topical agents against 200 strains of dermatophytes. J. Clinical Microbiology (2003) 41: 5209-5211.

(14) CLSI (2008). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard- Document M38-A. 2nd ed., Clinical and Laboratory Standards Institute, wayne pa (2004).

(15) Bueno JG, Martinez C and Zapata B. In-vitro activity of fluconazole,itraconazole,voriconazole and terbinafen against fungi as agent of onychomycosis . Clinical and Experimental Dermatology. (2010) 35: 658-663.

(16) Carrillo-Munoz AJ, Giussiano G, Guarro J, Quindos G, Guardia C, Valle O, Rodriguez V, Estivill D and Cardenes CD. In-vitro activity of voriconazole against dermatophytes , Scopulariopsis brevicaulis and other opportunistic fungi as agents of onychomycosis. International J. Antimicrobial Agents. (2007) 30: 157-161.

(17) Coelho L M, Ferreria RA, Leite Maffei CM and Martinez-Rossi NM. In-vitro antifungal drug susceptibilities of dermatophytes microconidia and arthroconidia. J. Antimicrobial Chemotherapy. (2006) 62: 758-761.

(18) Ghannoum M, Skaggs B and Sheehan D. Voriconazole susceptibilities of dermatophyte isolates obtained from a worldwide tinea capitis clinical trial. J. Clinical Microbiology. (2004) 44: 2579-2580

(19) Falahati M, Omidi Tabrizi N and Jahanbani F. Anti dermatophyte activities of Eucalyptus camaldulensis in comparison with griseofulvin. Iranian J. Pharm. Therapeutics. (2005) 4: 80-83.

(20) Shams-Ghalafsarohkhi M, Shokoohami MR, Amirrajab N, Moghadasi B, Ghajari A and Zeini F. In-vitro antifungal activities of Allium cepa,Allium sativum and ketoconazole against some pathogenic yeasts and dermatophytes. Fitoterapia. (2006) 77: 321-3.
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(21) Nowrozi H, Nazeri G, Adimi P, Bashashati Mand Emami M, Comparison of the activities of four antifungal agents in an in-vitro model of dermatophyte nail infection. Indian J. dermatology. (2008) 53: 125-128.

(22) Pakshir K, Bahedinie L, Rezaei Z, Sodafi Mand Zomorodian K, In-vitro activity of six antifungal drugs against clinically important dermatophytes. Jundishapur J. Microbiology. (2009) 2: 158-163.

(23) Mikaeli A, Modaresi M, Karimi I, Fathi Mand Jalilian N, Antifungal activities of Astragalus verrucosum on in-vitro and in-vivo guinea pig model of dermatophytosis. Mycoses. (2012) 55: 318-325.

(24) Chadeganipour M, Nilippour S, Havaaei A, in-vitro evaluation of griseofulvin against clinical isolates of dermatophytes from isfahan. Mycoses. (2004) 47: 503-507.

(25) Cassens BJ. Preventive Medicine and Public Health. 2nd ed, William and Wilkins, Baltimore, Maryland (1992) 45-46.

(26) Li RY, Wan Z, Wang AP, Shen YN, Lu CM, Li M, Xi LY, Liu WD, Zeng FQ, in-vitro susceptibility testing of amorolfine in pathogenic fungi isolated from dermatomycosis patients in china. Mycoses. (2004) 47: 402-406.

(27) Santos DA, Hamdan JS. In-vitro antifungal oral drug and drug combination activity against onychomycosis causative dermatophytes. Medical mycology. (2006) 44: 357-362.

(28) Galuppi R, Gambarara A, Bonoli C, Ostanello F and Tampieri MP. Antimycotic effectiveness against dermatophytes: comparison of two in-vitro tests. Vet Res Commun. (2010) 34: S57-S61.

(29) Esteban A, Abarca ML and Cabanes FJ. Comparison of disk diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes. Medical Mycology. (2005) 43: 61-66

(30) Silva BARROS ME, Santos DA and Hamdan JS. In-vitro methods for antifungal susceptibility testing of Trichophyton spp. Mycological Research. (2006) 110: 1355-1360.

(31) Gupta AK, Kohli Y. In-vitro susceptibility testing of ciclopirox, terbinafin, ketoconazole and itraconazole against dermatophytes and nondermatophytes and in-vitro evaluation of combination antifungal activity. British J. Dermatology. (2003) 149: 296-305.

(32) Mukherjee PK, Leidich SD, Isham N, Leitner I, Ryder NS and Ghannoum MA, Clinical Trichophyton rubrum strain exhibiting primary resistance to terbinafin. Antimicrobial Agents and Chemotherapy. (2003) 47: 82-86.

(33) Korting HC, Olert M and Abeck D. Results of German multicenter study of antimicrobial susceptibilities of Trichophyton rubrum and Trichophyton mentagrophytes strains causing tinea unguium. Antimicrobial Agent And Chemotherapy. (1995) 39: 1206-1208.

(34) Fernandez - Torres B, Inza I and Guarro J. Evaluation of disk diffusion method for determining eberconazole susceptibility of dermatophytes and influence of culture medium. Antimicrobial Agents And Chemotherapy. (2005) 49: 2116-2118.

(35) Mendez- Tovar LJ, Manzano- Gonzalez R, Velasquez- Hernandez V, Milan-Chiu B, Hernandez-Haernandaex F, Mondragon-Gonzalez R and Lopez-Martinez R. Resistance to azolic compounds in clinical Trichophyton spp. strains. Rev. Iberoam. Mycol. (2007) 24: 320-2.

(36) Santos DA and Hamdan JS. In-vitro activities of four antifungal drugs against Trichophyton rubrum isolates exhibiting resistance to fluconazole. Mycoses. (2007) 50: 286-289.

(37) Foroumadi A, Bolouri F, Moghami Tabrizi N, Davari Tanha F, Niroomand N, Azoodeh A, Emami S and Falahati M. Effectiveness of fluconazole for suppressive maintenance therapy in patients with RVVC: a randomized placebo- controlled study. Iran. J. Pharm. Res. (2009) 8: 307-313.

(38) Safari-nodoushan AA, Kazemi AH, Mirzaei F and Dehghani M. Fluconazole susceptibility profile of candida isolates recovered from patients specimens admitted to Yazd Central Laboratory. Iran. J. Pharm. Res. (2008) 7: 69-75.

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