In vitro antifungal susceptibility profile of 12 antifungal drugs against 55 *Trichophyton schoenleinii* isolated from tinea capitis favosa in Iran, Turkey and China

Running title: Antifungal susceptibility of *Trichophyton schoenleinii*

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Potential conflict of interest

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Abstract

Trichophyton schoenleinii is an anthropophilic dermatophyte mainly causing tinea favosa of the scalp in certain regions of the world, especially Africa and Asia. We investigated the *in vitro* susceptibilities of 55 *T. schoenleinii* collected over the last thirty years from Iran, Turkey and China against 12 antifungals using the CLSI broth-microdilution method. Our results revealed that terbinafine and ketoconazole were the most potent antifungal agents among those tested, independent of the geographical regions isolated.

Key words

Trichophyton schoenleinii, tinea capitis favosa, antifungal susceptibility testing

Favus or tinea capitis favosa, is a chronic inflammatory dermatophytosis of the scalp, particularly diagnosed in children, aged 4-14 years and occasionally in adults (1, 2, 3). Favus is characterized by scutula formation and scarring atrophy (cicatricial alopecia), which can be differentiated from other clinical forms of tinea capitis, e.g., tinea capitis superficialis and kerion Celsi (1, 4).

Anthropophilic *Trichophyton schoenleinii* is responsible for over 95% of favus cases (5). However, in rare instances, several anthropophilic (*T. violaceum*), zoophilic (*T. quinckeanum* and *T. verrucosum*), and geophilic (*Microsporum gypseum*) dermatophytes are reported as etiological agents of favus (1, 6).

With the introduction of griseofulvin in 1958, the anthropophilic agents of tinea capitis, *T. schoenleinii* and *M. audouinii*, were almost eradicated in most parts of the world (5-7). Currently, favus is common mainly in African countries; Nigeria (8) and Ethiopia (9), and Western China (5, 10), and geographic regions where lifestyles are associated with malnutrition and poverty (11, 12). The disease has also been reported sporadically in Iran (13), Turkey (14, 15), Western Europe (3) and South America (11).

Importantly the efficacy of griseofulvin has been decreased over the years, which now requires larger doses and longer treatment duration (16, 17). This suggests that Griseofulvin is no longer the treatment of choice in superficial cutaneous fungal infections (18, 19). In contrast, the newer antifungal drugs such as allylamine terbinafine, triazoles, and echinocandins have the advantage of shorter treatment durations than griseofulvin, and may remain present in fungicidal concentrations for several weeks after the course of treatment has been completed,

which allows short treatment duration with fewer side effects and also to prevent of re-infection (17, 18).

Although the infections caused by *T. schoenleinii* are of considerable medical importance, little is known on utility of the newer antifungal agents for the management of tinea capitis caused by *T. schoenleinii* from different geographical regions. Therefore, we investigated the *in vitro* susceptibilities of a large collection of clinical isolates of *T. schoenleinii* strains to 12 antifungals drugs by using the Clinical and Laboratory Standards Institute (CLSI) broth-microdilution method (20).

A total of 55 *T. schoenleinii* isolates obtained from patients with tinea capitis from Iran, Turkey and Western China were used. All isolates were cultured on Sabouraud glucose agar (Merck, Darmstadt, Germany) at 25 °C for 5 to 7 days. For identification, morphological identifications were confirmed using sequence-based analysis of the rDNA Internal Transcribed Spacer (ITS) regions, as described previously (21).

Conidial suspensions were harvested after isolates were sub-cultured on SDA for 5 to 7 days at 25°C and were suspended in normal saline containing 0.025% Tween 20. The inocula were then prepared spectrophotometrically and further diluted in normal saline in order to obtain a final inoculum concentration of 0.5-2.5 x 10^6 CFU/m.

We tested the *in vitro* susceptibility of the isolates against 12 antifungals by using a broth-microdilution format according to CLSI guidelines (20). Final concentrations of the following antifungal agents ranged from 0.016 to 16 microgram/ml: amphotericin B, ketoconazole, miconazole, itraconazole, voriconazole, posaconazole, caspofungin, anidulafungin, and terbinafine. Flucytosine, fluconazole, and griseofulvin were assessed over a 2-fold

concentration range, from 0.064 to 64 microgram/ml. The minimum inhibitory concentrations (MICs) of amphotericin B, flucytosine, ketoconazole, miconazole, fluconazole, itraconazole, voriconazole, posaconazole, griseofulvin, and terbinafine were determined visually: an inverted mirror was used for comparing the growth in wells containing the drugs with that in the drug-free control well. The results were also read using a microtitration plate spectrophotometric reader (Anthos htIII; Anthos Labtec Instruments, Salzburg, Austria). The minimum effective concentrations (MECs) of caspofungin and anidulafungin were read using a plate microscope (Olympus SZX9; Olympus Nederland, Zoeterwoude, The Netherlands), at 25× to 50× magnification.

The ranges and geometricmeans (GMs) of the MICs and MECs were determined for each species and drug after 48 and 72 h of incubation. If no growth was observed or growth was inadequate, incubation was extended to 120 h. *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019), and *C. krusei* (ATCC 6258) and *T. mentagrophytes* (ATCC MYA 4439), were used for quality controls in all experiments. All experiments on each strain were performed using three independent replicates on different days.

Data were analyzed using GraphPad Prism, Version 5.0, for Windows (GraphPad Software, San Diego, CA). MIC/MEC distributions between the groups and within distinct geographical areas were compared using Student's *t* test and the Mann-Whitney-Wilcoxon test; differences were considered statistically significant at P value of ≤ 0.05 (two-tailed).

The overall results obtained from visual and spectrophotometric readings were similar for the MIC and MEC endpoints. The geometric mean (GM) of MICs/MECs, the MIC/MEC ranges, the

153 MIC_{50}/MEC_{50} and MIC_{90}/MEC_{90} distributions of the 12 antifungals agents 55 *T. schoenleinii*
154 isolates are listed in Table 1.

155 The geometric means of the minimum inhibitory/effective concentrations (MICs/MECs) of the
156 antifungals across all isolates were the following (in increasing order): terbinafine (0.05
157 microgram/ml), posaconazole (0.20 microgram/ml), amphotericin B (0.29microgram/ml),
158 ketoconazole (0.52microgram/ml), miconazole (0.57microgram/ml), caspofungin (0.60
159 microgram/ml), anidulafungin (0.68microgram/ml), itraconazole (0.81 microgram/ml),
160 voriconazole (0.89 microgram/ml), griseofulvin (0.92 microgram/ml), fluconazole (25
161 microgram/ml), and flucytosine (> 64 microgram/ml).

162 The MIC/MEC ranges across all isolates were as follows: terbinafine (0.016-025 microgram/ml),
163 posaconazole (0.031-0.5 microgram/ml), amphotericin B (0.031-0.5 microgram/ml),
164 ketoconazole (0.125-1 microgram/ml), miconazole (0.125-1microgram/ml), caspofungin
165 (0.25-1 microgram/ml), anidulafungin (0.016-8microgram/ml), itraconazole (0.063-4
166 microgram/ml), voriconazole (0.063-4 microgram/ml), griseofulvin (0.05-2 microgram/ml),
167 fluconazole (4 - 64 microgram/ml), and flucytosine (64 - > 64 microgram/ml).

168 The highest MIC_{90} values were 64 microgram/ml, for flucytosine and fluconazole, which were
169 significantly different from those of the other 12 antifungal agents ($P < 0.01$). No statistically
170 significant differences in the susceptibility profiles of T. schoenleinii were detected within the
171 geographical regions investigated ($P > 0.05$).

172 Antifungal therapy is a central component of patient management for dermatophytosis, and
173 depending on the strategy chosen, topical and/or systemic drugs can be used (22). Despite
174 increasing number of investigations on utility of the newer antifungal agents for the

management of dermatophytosis (17, 23), the *in vitro* antifungal-susceptibility profiles of newer antifungal agents against *T. schoenleinii* remains poorly investigated. Most of the studies on the topic have only investigated a limited number of *T. schoenleinii* strains in the general context of testing the susceptibility of dermatophytes (24-30).

To the best of our knowledge, our study provides the first profiles of susceptibility to 12 antifungals using a large set of clinical *T. schoenleinii* strains isolated from tinea capitis favosa from a wide geographical range, worldwide. For all tested strains, terbinafine, posaconazole, amphotericin B, ketoconazole, miconazole, caspofungin, anidulafungin, itraconazole, voriconazole, griseofulvin, had low MICs values, whereas fluconazole and flucytosine did not show inhibitory effects.

Our study confirms those of previous studies, in which terbinafine demonstrated potent antifungal activity against dermatophyte species obtained from tinea capitis patients with the MIC ranging 0.02 to 0.13 microgram/ml (24, 25, 27-29).

With the exception of fluconazole, all tested azoles showed potent *in vitro* activity against *T. schoenleinii*. The activity of posaconazole (GM 0.20 microgram/ml, MIC range 0.031 to 0.5 microgram/ml) was of a similar level as terbinafine (GM 0.05 microgram/ml, MIC range 0.016 to 0.13 microgram/ml), and this was followed by the activity of ketoconazole (GM 0.52 microgram/ml, MIC range 0.125 to 1 microgram/ml), miconazole (GM 0.57 microgram/ml, MIC range 0.125 to 1 microgram/ml), itraconazole (GM 0.81 microgram/ml, MIC range 0.063 to 4 microgram/ml), and voriconazole (GM 0.89 microgram/ml, MIC range 0.063 to 4 microgram/ml).

In agreement with our finding, Fernandez-Torres also previously teste 2 *T. schoenleinii* strains and reported a itraconazole MIC range of 0.01−0.05 microgram/ml, voriconazole MIC range of

0.01−0.06 microgram/ml, miconazole MIC range of 0.031−0.063 microgram/ml, ketoconazole MIC range of 0.03−0.125 microgram/ml, and fluconazole MIC range of >16 microgram/ml, respectively (24). In another study by Indira et al (28), ketoconazole and itraconazole also demonstrated MIC range of 0.06 to 0.96 mg/ml and 0.12 to 0.96 mg/ml, respectively. Similarly, few other studies also have reported potent *in vitro* activity of azoles against *T. schoenleinii* (25-27, 29, 30).

In the present study, amphotericin B was potently effective (MIC range of 0.031−0.5 microgram/ml) against all 55 *T. schoenleinii* strains tested, which agrees with previous reports of an amphotericin B MIC of 0.25 microgram/ml (24). In our study, we also observed that MEC values of caspofungin and anidulafungin were relatively low, with MEC range of 0.25 to 1 and 0.016 to 8 microgram/ml, respectively.

Of all agents tested, fluconazole and flucytosine were the drugs for which the highest MIC values were measured, which is similar to the results of previous studies (24). No clinical investigation has been conducted using flucytosine and dermatophytes, but fluconazole has been used for treating tinea capitis. Previous studies have shown that high doses of fluconazole (≥4 - 8 mg/kg/week) applied for long durations (12–16 weeks) might be used for treatment of tinea capitis regardless of the fungus type (18).

Although, for almost four decades griseofulvin was the standard treatment for tinea capitis, worldwide (18), nowadays it is no longer the treatment of choice in superficial cutaneous fungal infections (18). The efficacy of griseofulvin has decreased over the years, resulting to griseofulvin-resistant isolates of dermatophytes (31). It now requires larger doses and longer treatment duration, which put the patent at the higher toxicity risk (16).

In conclusion, our results revealed that terbinafine and ketoconazole were the most potent antifungals against *T. schoenleinii* among systemic and topical antifungals tested, independent of geographical regions isolated. However, it will be necessary to obtain more clinical data to confirm if this potent *in vitro* efficacy is predictive for clinical outcome.

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	AmB	5-FC	FLC	ITC	VRC	POS	MCZ	KTZ	AFG	CAS	GRZ	TBF
Range	0.031-0.5	64 - > 64	4-64	0.063-4	0.063-4	0.031-0.5	0.125-1	0.125-1	0.016-8	0.25-1.00	0.05-2	0.016-0.125
MIC_{50}/MEC_{50}	0.25	64	16	0.25	0.25	0.125	0.50	0.50	0.02	0.5	0.63	0.031
MIC_{90}/MEC_{90}	0.5	64	64	2	2.00	0.50	1.00	1.00	0.02	1.00	2	0.125
Geometric mean	0.29	64	25	0.81	0.89	0.20	0.57	0.52	0.68	0.60	0.92	0.05

Table 1. Geometric mean of MICs/MECs, MIC/MEC ranges, and MIC_{50}/MEC_{50} and MIC_{90}/MEC_{90} values obtained by testing the susceptibility of 55 *Trichophyton schoenleinii* strains to 12 antifungal agents.

MIC: Minimum inhibitory concentration.

MEC: Minimum effective concentration.

AmB: amphotericin B, 5-FC: flucytosine , FLZ: fluconazole, ITC: itraconazole, VRC: voriconazole, POS: posaconazole, MCZ: miconazole, KTZ: ketoconazole, AFG: anidulafungin, CAS: caspofungin, GRZ: griseofulvin, TBF: terbinafine.