In vitro antifungal susceptibility of *Trichophyton violaceum* isolated from tinea capitis patients


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**Objectives:** *Trichophyton violaceum* is an anthropophilic dermatophyte that is endemic to parts of Africa and Asia and is sporadic in Europe. *T. violaceum* mainly causes tinea capitis in both children and adolescents. Although the infections caused by *T. violaceum* are of considerable medical importance, its antifungal susceptibility profile remains poorly examined.

**Methods:** In this study, we tested the in vitro antifungal susceptibility of a set of clinical *T. violaceum* isolates obtained from tinea capitis patients, using the CLSI broth microdilution method. We tested eight antifungals and used isolates collected from Western China (21), Eastern China (12), the Middle East (1), Europe (20), South Africa (7) and Canada (1).

**Results:** The geometric means of the MICs of the antifungals for all isolates were as follows (in increasing order): posaconazole, 0.021 mg/L; terbinafine, 0.023 mg/L; voriconazole, 0.062 mg/L; amphotericin B, 0.20 mg/L; itraconazole, 0.34 mg/L; caspofungin, 0.56 mg/L; fluconazole, 4.23 mg/L; and flucytosine, 8.46 mg/L. No statistically significant differences in the susceptibility profiles of *T. violaceum* were detected within the geographical regions tested.

**Conclusions:** Posaconazole, terbinafine and voriconazole were shown to be the most potent antifungal agents against *T. violaceum* isolates obtained from tinea capitis patients worldwide. These results might help clinicians in developing appropriate therapies that have a high probability of successfully treating tinea capitis due to *T. violaceum*.

**Keywords:** *T. violaceum*, antifungal susceptibility testing, fungus

**Introduction**

*Trichophyton violaceum* is an anthropophilic dermatophyte causing mainly tinea capitis, a fungal infection of the scalp occurring particularly in children and adolescents, 1, 2 and occasionally tinea corporis. The fungus is endemic to the arid climate zones of northern Africa, Eastern Europe 3 and large parts of Asia, 4, 5 where tinea capitis remains a major public health problem among schoolchildren in rural areas. In the temperate zones of Europe and North America, *T. violaceum* is an emerging pathogen because of immigration from endemic areas. 6 Notably, although the typical dermatophyte profile has undergone a change as a result of recent socioeconomic changes and migration, *T. violaceum* has remained the primary species responsible for tinea capitis for several decades in Xinjiang, a province in Western China. 4

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Although the infections caused by T. violaceum are of considerable medical importance, its antifungal susceptibility profile remains poorly examined. Most of the studies on the topic have only investigated a limited number of T. violaceum strains in the general context of testing the susceptibility of dermatophytes. Therefore, we investigated the in vitro susceptibilities of a large collection of clinical isolates of T. violaceum strains to eight antifungal drugs by using the CLSI broth microdilution method.

Materials and methods

Fungal strains

We used 62 clinical isolates, 50 of which were obtained from patients with tinea capitis and 12 were derived from skin or nail. We collected 21 isolates from children with symptomatic tinea capitis in Xinjiang province in Western China and 12 isolates from Jiangxi province in Eastern China; we also included 20 strains from Europe, 7 from Africa, 1 from Iran and 1 from Canada. Each of the fungal strains was obtained from a different patient. The identities of the strains were confirmed down to the species level by sequencing the ribosomal DNA (rDNA) internal transcribed spacer (ITS) regions.

Antifungal susceptibility testing

A previously described method was used to obtain the conidia used for susceptibility testing. We tested the in vitro susceptibility of the isolates to eight antifungals by using a broth microdilution format according to CLSI guidelines. The MICs of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, caspofungin, posaconazole, and terbinafine were determined visually: an inverted mirror was used for comparing growth in wells containing the drugs with that in the drug-free control well. The minimum effective concentrations (MECs) of caspofungin were read using a plate microscope (Olympus SZX9; Olympus Nederland, Zoeterwoude, the Netherlands), at ×25 magnification. The results were also read using a microtitration plate spectrophotometric reader (Anthos htIII; Anthos Labtec Instruments, Salzburg, Austria). Paecilomyces variotii (ATCC 6258) was used as a quality control.

Statistical analysis

Data were analysed using GraphPad Prism, Version 5.0, for Windows (GraphPad Software, San Diego, CA, USA). MIC/MEC distributions between 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 a P value of ≤0.05 (two-tailed).

Results

The overall results obtained from visual and spectrophotometric readings were similar for the MIC and MEC endpoints after 72 and 120 h of incubation. The GM values of MICs (mg/L) for all isolates used in this study were as follows (in increasing order): posaconazole, 0.021; terbinafine, 0.023; voriconazole, 0.062; amphotericin B, 0.20; itraconazole, 0.34; caspofungin, 0.56; fluconazole, 4.23; and flucytosine, 8.46.

As shown in Table 1, posaconazole and terbinafine had the highest in vitro activity against all strains; their MICs ranged from 0.016 to 0.064 mg/L and 0.016 to 0.064 mg/L, respectively. The MIC50 (minimal concentration that inhibits 50% of isolates) and MIC90 (minimal concentration that inhibits 90% of isolates) values were calculated for species with ≥10 isolates from the same geographical region.
from 0.016 to 0.064 mg/L and from 0.016 to 0.125 mg/L, respectively. The MIC ranges of other agents were as follows: voriconazole, 0.016–0.125 mg/L; amphotericin B, 0.064–0.25 mg/L; and itraconazole, 0.016–1 mg/L.

The widest MIC ranges were measured for fluconazole (0.5–16 mg/L) and flucytosine (2–16 mg/L). The caspofungin MIC range was 0.125–1 mg/L. The highest GM MIC90 values were 8 mg/L, for fluconazole, followed by 16 mg/L, for flucytosine, which were significantly different from those of the other six antifungal agents (P<0.01).

Furthermore, our results indicated that no statistically significant differences existed in the patterns of susceptibility to each compound within the geographical regions studied.

Discussion

The in vitro antifungal susceptibility profile of T. violaceum remains poorly investigated. To the best of our knowledge, our study provides the first profiles of susceptibility to eight antifungals using a large set of clinical T. violaceum strains isolated from a wide geographical range of tinea capitis and other infections. For all tested strains, posaconazole, terbinafine, voriconazole, amphotericin B, itraconazole and caspofungin had low MICs, whereas fluconazole and flucytosine did not exert inhibitory effects.

In this study, among the antifungal drugs tested, posaconazole and terbinafine had the lowest MICs for T. violaceum. Our findings confirm those of previous studies, in which terbinafine demonstrated potent antifungal activity against dermatophyte species obtained from tinea capitis patients worldwide. Fernandez-Torres and others conducted a study using seven T. violaceum strains and reported a terbinafine MIC range of 0.007–0.125 mg/L, a GM MIC of 0.01 mg/L and an MIC90 of 0.03 mg/L. Similarly, in the study by Ghannoum and others, the MICs ranged from 0.01 to 0.125 mg/L in a set of 63 isolates obtained from tinea capitis worldwide. In agreement with the in vitro data, terbinafine has been widely reported to elicit a strong clinical response against Trichophyton species, the cure rate being ≥80%.

Azole agents exert their antifungal activity by blocking the demethylation of lanosterol and thereby inhibiting ergosterol synthesis. In our study, the activities of posaconazole and terbinafine were at a similar level, higher than the other antifungals used, and this was followed by the activity of voriconazole. Similarly, in a previous study Singh and others reported a posaconazole MIC range of 0.03–1 mg/L and a GM MIC of 0.32 mg/L. Findings similar to ours were also obtained for voriconazole (MICs, 0.002–0.06 mg/L) using T. violaceum isolates obtained from a worldwide tinea capitis clinical trial. We also found that the MIC values of itraconazole against all T. violaceum isolates were very low (0.016–1 mg/L). To date, no clinical study has been conducted using posaconazole and voriconazole to treat tinea capitis, although review of the literature suggests that itraconazole offers an alternative to griseofulvin for the treatment of kerion and non-inflammatory tinea capitis.

Polyene agents exert their antifungal activity by binding to ergosterol in the fungal cell membrane. This disrupts cell permeability and results in rapid cell death. In our study, amphotericin B was also potently effective against T. violaceum, which agrees with previous reports of an amphotericin B MIC90 of 0.25 mg/L. However, the MIC values are lower than those measured using other dermatophyte species, such as Trichophyton mentagrophytes (MIC90, 0.5 mg/L) and Epidermophyton floccosum (MIC90, 1 mg/L), demonstrating a 2 log2 dilution step difference.

Echinocandins function by inhibiting the production of (1,3)-β-D-glucan, an essential component of the fungal cell wall. In our study, the MEC values of caspofungin were low (GM, 0.56 mg/L; MEC90, 1 mg/L); this is comparable to the results of Bao and others (GM, 0.871 mg/L; MEC90, 1 mg/L) and demonstrates a 1 log2 dilution step difference.

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. 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) are required regardless of the fungus type.

Of note, T. violaceum is one of the most common dermatophytes that cause endothrix-type hair invasion, with the fungus entering the cortex just above the hair bulb and encircling the shaft beneath an intact cuticle. Given that the hair shaft is not accessible by topical antifungals, treatment of endothrix requires a long duration (>4 weeks) of systemic antifungal use. For many years, griseofulvin was the standard treatment for tinea capitis. However, in our current study we did not investigate griseofulvin. Due to a number of factors, including changes in epidemiology and genetic mutations in the infective fungi resulting in decreased susceptibility, the efficacy of griseofulvin has decreased over the years and now requires larger doses and longer treatment duration. This suggests that it is no longer the treatment of choice in superficial cutaneous fungal infections. In contrast, the newer antifungal drugs 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 and prevention of re-infection.

In conclusion, posaconazole, terbinafine and voriconazole were shown to be the most potent antifungal agents against T. violaceum isolates obtained from tinea capitis patients worldwide. These results might help clinicians in developing appropriate therapies that have a high probability of successfully treating tinea capitis. However, further clinical investigations must be conducted in order to develop interpretive breakpoints.

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References