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Voriconazole Susceptibilities of Dermatophyte Isolates Obtained from a Worldwide Tinea Capitis Clinical Trial

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In this study, the voriconazole susceptibilities of dermatophyte isolates obtained from a worldwide tinea capitis trial were compared to their susceptibilities to fluconazole and griseofulvin. The MIC ranges of voriconazole, fluconazole, and griseofulvin, were 0.002 to 0.06 μg/ml, 0.25 to 32 μg/ml, and 0.125 to 2.0 μg/ml, respectively.

Tinea capitis, a dermatophyte infection of the scalp caused mainly by *Trichophyton* and *Microsporum* species, remains common among the pediatric population. Recently, this disease has been recognized as an important public health problem in the United States with 13% of school children, especially those of African-American descent, testing positive for dermatophytes (6). The traditional antifungal agent for the treatment of tinea capitis has been griseofulvin, which is currently the only drug approved by the FDA for this application. However, compliance with griseofulvin therapy is generally low because of unpleasant taste (4) and over the years, higher doses and longer courses of treatment with this agent have been required for a successful outcome (5, 9). Thus, there is a need for more effective agents to treat tinea capitis.

To date, our knowledge of the susceptibility patterns of dermatophytes causing tinea capitis is lacking, possibly because a reference method to determine the antifungal susceptibility of dermatophytes has not been established until recently. Under the auspices of the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS), our group, in collaboration with seven other laboratories, developed a method to determine the susceptibility of dermatophytes to various antifungals (8, 10) and has shown that this method has good inter- and intralaboratory agreement (7).

In this study, the voriconazole susceptibility profile of the baseline isolates ($n = 817$) collected from subjects enrolled in a large multinational tinea capitis clinical trial was determined by this new method. Patients enrolled in the trial come from different geographical regions of the world, including the United States, Puerto Rico, Guatemala, Chile, Costa Rica, and India. As expected, *Trichophyton tonsurans* was the predominant dermatophyte isolated from patients from U.S. sites. Isolates from Central and South American sites were predominantly *Microsporum canis*, while those from India were predominantly *Trichophyton violaceum*.

All isolates were identified to the genus and species levels by colonial and microscopic characteristics, as well as standard biochemical tests. Isolates, including *T. tonsurans* ($n = 718$), *T. violaceum* ($n = 13$), *Trichophyton mentagrophytes* ($n = 1$), *M. canis* ($n = 83$), and *Microsporum gypseum* ($n = 2$), were frozen at −80°C and batched for susceptibility testing. Isolates were subcultured onto potato dextrose agar (Fisher Scientific, Hampton, NH) and incubated at 30°C until good conidiation was achieved, usually within 7 days. *T. violaceum* isolates characteristically form compact colonies with numerous chlamydospores and no conidia. In order to obtain conidia for susceptibility testing, we used the method of Ogasawara et al. (11), incubating the *T. violaceum* colonies for 6 weeks or longer until conidium-bearing white fluffy colonies appeared on the surface of the original growth. Conidia were harvested to sterile saline by swabbing the colony surface with a sterile swab and were allowed to settle for 10 to 15 min. Conidium counts were standardized with a hemacytometer, and the suspension was adjusted to 1×10^3 to 3×10^3 conidia/ml in RPMI 1640 medium buffered with MOPS [3-(*N*-morpholino)propanesulfonic acid; Hardy Diagnostics, Santa Maria, CA]. Antifungal powders were reconstituted and serial dilutions were prepared in accordance with CLSI M38A methodology (3). Serial dilutions of drug (0.001 to 0.5 μg/ml for voriconazole and 0.125 to 64 μg/ml for fluconazole and griseofulvin) and inoculum were combined in 96-well round-bottom microtiter plates and incubated at 35°C for 4 days. The MIC endpoint was defined as the lowest concentration to inhibit 80% of fungal growth compared to the growth control. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as controls.

Table 1 summarizes the MIC ranges, MIC_{50}s, and MIC_{90}s of each antifungal by geographic area. The voriconazole MIC range for all isolates tested was 0.002 to 0.06 μg/ml, and the voriconazole MIC_{50} and MIC_{90} were 0.015 and 0.03 μg/ml, respectively. The fluconazole MIC range, MIC_{50}, and MIC_{90} were 0.25 to 32, 4.0, and 8.0 μg/ml, respectively, while the griseofulvin MIC range, MIC_{50}, and MIC_{90} were 0.125 to 2.0, 0.5, and 1.0 μg/ml, respectively. Comparison of the MIC data for U.S. isolates showed that these values in general were similar to those for non-U.S. isolates, with a few isolates differing by 1 to 2 dilutions. Similar agreement was seen with the MIC_{50}s and MIC_{90}s, which were also within two dilutions for each species. There exists an inherent 1-dilution variation in

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TABLE 1. MIC data by geographic distribution

Geographic distribution (no. of isolates)	MIC (μg/ml) of:								
	Voriconazole			Fluconazole			Griseofulvin		
	Range	50% of strains	90% of strains	Range	50% of strains	90% of strains	Range	50% of strains	90% of strains
United States and Puerto Rico (752)	0.002–0.06	0.015	0.03	0.25–16	4.0	8.0	0.125–2.0	0.5	1.0
Central and South America (51)	0.008–0.06	0.03	0.06	1.0–8.0	4.0	8.0	0.125–0.5	0.25	0.5
India (14)	0.015–0.06	0.03	0.03	2.0–32	8.0	16	0.25–2.0	1.0	2.0
All dermatophytes	0.002–0.06	0.015	0.03	0.25–32	4.0	8.0	0.125–2.0	0.5	1.0

MIC microdilution testing, and a 2-dilution difference meets the generally accepted criteria for agreement (1, 2).

In conclusion, our data showed that (i) voriconazole demonstrated potent antifungal activity against all isolates and (ii) the voriconazole susceptibility of dermatophyte isolates obtained from U.S. sites was similar to that from non-U.S. sites, indicating that there is no difference in voriconazole susceptibility within the dermatophyte species obtained worldwide. However, to confirm this conclusion, a larger number of dermatophytes from non-U.S. sites should be tested. The susceptibilities of fluconazole and griseofulvin were also similar among geographic locations.

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