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Comparison of the *In Vitro* Activities of Newer Triazoles and Established Antifungal Agents against *Trichophyton rubrum*

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One hundred eleven clinical *Trichophyton rubrum* isolates were tested against 7 antifungal agents. The geometric mean MICs of all isolates were, in increasing order: terbinafine, 0.03 mg/liter; voriconazole, 0.05 mg/liter; posaconazole, 0.11 mg/liter; isavuconazole, 0.13 mg/liter; itraconazole, 0.26 mg/liter; griseofulvin, 1.65 mg/liter; and fluconazole, 2.12 mg/liter.

Dermatophytosis caused by *Trichophyton rubrum* is the most common cutaneous fungal infection worldwide (1), which represents the cause of between 80% and 90% of all chronic and recurrent infections (2). These infections establish an important public health problem because of the prolonged treatment required for the disease, because of the frequent recurrence of infection, and because they are generally considered difficult to manage (3). Reliable *in vitro* susceptibility testing would therefore be useful for selecting the most suitable antifungal treatment. For many years, griseofulvin was the only approved systemic antidermatophytic agent (4). However, nowadays, many potent antifungal agents are available for the treatment of dermatophytosis, such as allylamines and triazoles, which have more potent activity and fewer side effects (5–19). The expansion of information on *in vitro* susceptibility testing of dermatophytes to new antifungal agents will help in the selection and development of antifungal drug regimens.

The aim of the current study was to compare *in vitro* the activities of three newer triazoles, voriconazole, posaconazole, and isavuconazole, and four established antifungal agents against *T. rubrum* infection. One hundred eleven clinical isolates of *T. rubrum* were collected from seven dermatology clinics in Shanghai, China. Morphological identifications were confirmed by sequence-based analysis of the internal transcribed spacer of the rRNA gene region. The *in vitro* activities of seven antifungal agents were determined according to the CLSI reference guideline M38-A2 (20), with minor modifications. Two reference strains, *Trichophyton mentagrophytes* (strain ATCC MYA-4439) and *Candida parapsilosis* (strain ATCC 22019), were included as quality controls. Student's *t* test with the statistical SPSS package (version 9.0) was used, and *P* values of <0.05 were considered statistically significant.

Table 1 lists the MIC ranges, geometric mean (GM) MICs, MIC_{50}s, and MIC_{90}s of seven antifungal agents against 111 *T. rubrum* strains. Terbinafine, voriconazole, posaconazole, isavuconazole, itraconazole, and griseofulvin had low MICs against all tested strains, whereas fluconazole did not show inhibitory effects. Similar results have been achieved in other studies (Table 2); however, limited data are available for the newer triazoles isavuconazole and posaconazole.

Terbinafine was one of the most effective antifungal agents against *T. rubrum* among the 7 fungal agents tested, and our findings confirm those of previous studies (5–19) (Table 2).

TABLE 1 Geometric mean MICs, MIC ranges, MIC_{50}s, and MIC_{90}s obtained by susceptibility testing of antifungal agents against 111 *T. rubrum* clinical isolates

Drug	MIC/MEC (mg/liter)			
	Range	50%	90%	Geometric mean
Griseofulvin	1–4	2	2	1.65
Fluconazole	0.125–64	2	64	2.12
Itraconazole	0.031–16	0.5	2	0.26
Voriconazole	0.031–16	0.031	0.125	0.05
Posaconazole	0.016–1	0.125	0.5	0.11
Isavuconazole	0.031–4	0.06	0.125	0.13
Terbinafine	0.008–0.06	0.031	0.06	0.03

We compared the *in vitro* activities of the 3 newer triazoles isavuconazole, posaconazole, and voriconazole with that of itraconazole. Three newer triazoles offered good *in vitro* activity against *T. rubrum* (Table 1). All isolates were far more susceptible to the 3 newer triazoles than to itraconazole (Table 1) and comparable to those reported by other studies (7, 9, 10, 14, 17, 18).

Isavuconazole is a novel broad-spectrum triazole agent and has the same mechanism of action as the other triazoles. Several studies have supported its efficacy in invasive *Candida* species, *Cryptococcus neoformans*, *Aspergillus* species, and *Mucorales* isolates

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TABLE 2 Summarized data on susceptibility of *T. rubrum* to antifungal drugs in different studies from 2000 to 2014

Method for testing	MICs (mg/liter) for:															Reference
	Terbinafine		Itraconazole		Voriconazole		Fluconazole		Posaconazole		Griseofulvin		No. of strains	Incubation time[a]	Medium[b]	Incubation temp (°C)	
	Range	GM[c]	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM					
M38-A2	0.008 to 0.06	0.03	0.031 to 16	0.26	0.031 to 16	0.05	0.125 to 64	2.12	0.016 to 1	0.11	0.008 to 0.06	0.03	111	96	PDA	28	This paper
M38-A2	0.004 to 0.06	0.009	0.015 to 0.125	0.037									130	96	OA	30	5
M38-A2	0.007 to 0.5	0.04	0.031 to 1	0.1							0.03 to 2	0.08	78	96–120	OA	30	6
M38-A	0.0156 to 16	0.172	0.0009 to 4	0.06	0.0078 to 8	0.19	0.0625 to 256	11.05			0.0312 to 256	1.61	89	>168	PDA	28	7
M38-A	0.0075 to 0.015	0.01	0.062 to 16	0.24							0.5 to 2	0.48	23	120	PDA	28	8
M38-A					0.01 to 1	0.06	0.06 to 64	2.79					139	48, 72, 96, or longer	PDA	28	9
M38-A	0.001 to 0.03	0.006	0.25 to 2	0.59			0.5 to 64	1.92	0.06 to 1	0.21	0.5 to 1	0.88	16	120	PDA	30	10
M38-A	<0.007 to 0.031		0.015 to 0.25				1 to 64						50	168	PDA	28	11
M38-P	<0.008 to 0.015	0.0057	0.008 to 0.12	0.022			0.03 to 2	0.51					39	96	PDA	35	12
M38-A	<0.031		<0.031 to 1				>64				0.25 to 2.0		32	168	PDA	28	13
M38-P	0.003 to >2	0.02	0.015 to >8	0.07			0.125 to >64	5.36	0.007 to 0.5	0.05			73	168	OA	35	14
M27-A	0.003 to 1	0.003	0.06 to 32	0.14									68	168	OA	35	15
M38-P	0.01 to 0.06	0.03	0.06 to 2	0.42									10	168	PDA	28	16
M38-P	0.003 to >16	0.01	0.01 to 8	0.09	0.01 to 1	0.06	0.06 to >64	2.8					144	96	PDA	28	17
M27-A	<0.04 to 0.25	0.01	0.03 to 1	0.08	<0.125 to 1	0.38	2 to 8	3.31			0.5 to 8	1.95	27	12–21 days	PDA		18
M38-P	<0.0039 to 0.25	0.01	0.03 to 2	0.16									100	168	PDA	28	19

[a] Incubation time is in hours, unless otherwise stated.
[b] PDA, potato dextrose agar; OA, oatmeal agar.
[c] GM, geometric mean.

(5–19, 21). However, the antifungal susceptibility profile of dermatophytes remains poorly examined. Ghannoum and Isham reported that isavuconazole had shown potent *in vitro* activity against dermatophytes (22) and was more active than other triazoles tested (itraconazole and voriconazole), but it had a higher MIC than that of terbinafine; however, against *T. rubrum* isolates with high MICs to terbinafine, the isavuconazole MICs remained low (0.06 mg/liter for all tested isolates) (23). In our study, the MICs of isavuconazole (GM, 0.13 mg/liter; MIC_{90}, 0.125 mg/liter) were similar to those of posaconazole (GM, 0.11 mg/liter; MIC_{90}, 0.5 mg/liter) and voriconazole (GM, 0.05 mg/liter; MIC_{90}, 0.125 mg/liter); the difference was within 1 $\log_2$-dilution step, which was much lower than those of itraconazole (GM, 0.26 mg/liter; MIC_{90} 2 mg/liter) for the majority of the *T. rubrum* isolates tested.

Posaconazole showed activity similar to that described by Gupta, Kohli, and Batra (14), who reported posaconazole to be the most active compound, with an MIC_{90} of ≤1.0 mg/liter; the MIC_{90} was 0.5 mg/liter in our study. Similar data were reported by Singh, Zaman, and Gupta (10); however, the MIC was greater than that reported by us and Gupta, Kohli, and Batra (14). This variation may be a result of the different methods used (Table 2). The potent activity of posaconazole against *Trichophyton violaceum* (*T. rubrum* complex) has been reported by us as well (24).

The excellent activity of voriconazole against *T. rubrum* has been observed by B. Fernández-Torres et al. (17) and A. J. Carrillo-Muñoz et al. (9), with sample sets of 144 and 139 isolates, respectively (GM for both, 0.06 mg/liter). Our findings with 111 isolates have confirmed this good activity (GM, 0.05 mg/liter). There were, however, some discrepancies; in two of the previous reports, voriconazole appeared to be less active than itraconazole (7, 18). This could be attributed, at least partially, to the different methodology employed and the lack of standardized protocols. Our previous study (24) revealed that voriconazole had potent activity against *T. violaceum*.

For itraconazole, significant variations are shown in the published literature (Table 2). Overall, the geometric mean MIC of itraconazole for half of the isolates was <0.1 mg/liter, and the highest GM was 0.59 mg/liter (16), followed by 0.42 mg/liter (8). Our results showed good *in vitro* activity of itraconazole against *T. rubrum* (GM, 0.26 mg/liter); however, itraconazole was less active than the three new triazoles tested.

Griseofulvin was the first-line antifungal agent for the treatment of dermatophytoses for many years, but today, it is not widely used (4), due to griseofulvin-resistant isolates of dermatophytes and the existence of strains with elevated MICs to griseofulvin (6, 25–27). With our results, the MICs of griseofulvin for *T. rubrum* were in agreement with those reported by Adimi et al. (7) and Perea et al. (18). Griseofulvin was less active than the rest of the agents tested except for fluconazole against *T. rubrum*. Nevertheless, all strains were more susceptible to griseofulvin than to fluconazole (Table 1).

Among the studies reported in Table 2, fluconazole also was effective against *T. rubrum*, except in a study by Adimi et al. (7). Of all the agents tested in the current study, fluconazole showed the lowest activity, which was consistent with previous studies (9, 10, 16); although *T. rubrum* is not susceptible to fluconazole, it is recommended for the management of some dermatophytoses (28–30).

In conclusion, terbinafine, voriconazole, posaconazole, and isavuconazole were shown *in vitro* to be the most potent antifun-

gal agents against the *T. rubrum* isolates investigated. These results might help clinicians to develop appropriate therapies for treating dermatophytosis caused by *T. rubrum*. However, further clinical investigations must be conducted in order to develop interpretive breakpoints.

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