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In Vitro Activities of 10 Antifungal Drugs against 508 Dermatophyte Strains

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We have tested 508 strains belonging to 24 species of dermatophytes against 10 antifungal drugs following mainly the NCCLS (M38-P) standard for filamentous fungi. However, several important factors, such as the temperature (28 versus 35°C) and time of incubation (4 to 10 days versus 21 to 74 h), have been modified. The antifungals used were itraconazole, ketoconazole, miconazole, clotrimazole, voriconazole, terbinafine, amphotericin B, fluconazole, UR-9825, and G-1. In general, with the exception of fluconazole and G-1, all antifungals were shown to be highly effective.

Dermatophytes are a specialized group of fungi which affect keratinous tissue of humans and of other vertebrates, causing superficial infections. In recent years the number of infections caused by these fungi has increased considerably (7, 15), causing particular concern when they infect immunocompromised patients where atypical manifestations and more severe, extensive lesions can be produced (1, 14). Dermatophytoses generally respond well to topical antifungal therapy, although local therapy may be inappropriate for extensive infections or for infections affecting the nails or scalp. In recent years, a number of safe and highly effective antifungal agents have been introduced into clinical practice. Among them, terbinafine (TF), itraconazole (ITZ), fluconazole (FCZ), and more recently, voriconazole (VCZ) and the new triazole UR-9825, still under clinical investigation, are probably the most promising. However, their activity against significant number of strains, representing a wide spectrum of dermatophyte species and following standard procedures, has not yet been investigated. Consequently, the aim of this study has been to evaluate the in vitro activity of the traditionally available antifungal drugs and of some of the newer ones against a significant number of strains of dermatophytes by following mainly the NCCLS guidelines for testing filamentous fungi (11).

MATERIALS AND METHODS

Strains. A total of 508 strains of dermatophytes belonging to 24 species were tested. They were *Epidermophyton floccosum* ($n = 22$), *Microsporum audouinii* ($n = 8$), *Microsporum canis* ($n = 105$), *Microsporum cookei* ($n = 1$), *Microsporum ferrugineum* ($n = 4$), *Microsporum fulvum* ($n = 1$), *Microsporum gallinae* ($n = 1$), *Microsporum gypseum* ($n = 32$), *Microsporum nanum* ($n = 1$), *Microsporum praecox* ($n = 1$), *Microsporum racemosum* ($n = 1$), *Trichophyton ajelloi* ($n = 2$), *Trichophyton balcaneum* ($n = 2$), *Trichophyton concentricum* ($n = 2$), *Trichophyton erinacei* ($n = 7$), *Trichophyton interdigitale* ($n = 21$), *Trichophyton mentagrophytes* ($n = 122$), *Trichophyton phaseoliforme* ($n = 1$), *Trichophyton rubrum* ($n = 144$), *Trichophyton schoenleinii* ($n = 2$), *Trichophyton simii* ($n = 2$), *Trichophyton tonsurans* ($n = 18$), *Trichophyton verrucosum* ($n = 1$), and *Trichophyton violaceum* ($n = 7$). The majority of strains were clinical isolates from different hospitals in Spain and the United Kingdom, and numerous reference strains from the Centraalbureau voor Schimmelcultures were also tested. The fungi were maintained in sterile distilled water at room temperature (23 to 25°C) and prior to testing were subcultured on antimicrobial-agent-free potato dextrose agar (PDA) (Pronadisa, Madrid, Spain) at 28°C for 7 to 15 days to ensure the purity and viability of the inoculum. The strain *Paecilomyces variotti* (ATCC 36257) was included as quality control.

Medium. The tests were performed in RPMI 1640 medium (GIBCO BRL, Barcelona, Spain) with L-glutamine and without sodium bicarbonate buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma, Madrid, Spain).

Antifungal agents. The drugs were obtained from their respective manufacturers: amphotericin B (AMB) (E. R. Squibb & Sons, Barcelona, Spain); clotrimazole (CTZ) (Química Farmaceútica, Bayer, Barcelona, Spain); ITZ, ketoconazole (KTZ), and miconazole (MCZ) (Janssen Research Foundation, Beerse, Belgium); VCZ and FCZ (Pfizer, Madrid, Spain); G-1 (Centro de Bioactivos Químicos, Universidad Central de las Villas, Santa Clara, Cuba); TF (Novartis, Basel, Switzerland); and UR-9825 (J. Uriach & Co., S. A., Barcelona, Spain). All stock solutions were prepared in 100% dimethyl sulfoxide at a concentration of 1,600 μg/ml, with the exception of FCZ, which had a stock concentration of 6,400 μg/ml. Stocks were frozen at −20°C until needed. Serial drug dilutions were performed as described for the NCCLS reference method (11), beginning at 100 times the test concentration followed by a further 1:50 dilution in RPMI medium to yield twice the final concentration required for testing

Method. Tests were performed using a broth microdilution method mainly following the NCCLS guidelines (11) described in a previous study (3).

Inoculum preparation. Stock inoculum suspensions of the fungi were prepared from 7- to 15-day-old cultures grown on PDA at 28°C. Mature colonies were covered with approximately 10 ml of sterile saline (0.85%) by scraping the surface with the tip of a Pasteur pipette. The resulting mixture of conidia and hyphal fragments was withdrawn and transferred to sterile tubes. Heavy particles were allowed to settle for 15 to 20 min at room temperature; the upper suspension was mixed with a vorter mixer for 15 s. The turbidity of the supernatants was measured spectrophotometrically at a wavelength of 530 nm, and transmission was adjusted to 65 to 70%. Each suspension was diluted 1:50 in RPMI 1640 to obtain the final test inoculum twice. The inocula corresponding to 117 strains (24 species) (Table 1) were quantified by plating 0.01 ml of a 1:100 dilution of the adjusted inoculum on PDA plates. The plate contents were incubated then at 28°C and were observed daily for the presence of fungal colonies. Colonies were counted, when the growth became visible, as number of CFU per milliliter.

Test procedure. The tests were performed in sterile, round-bottomed, 96-well microplates (Soria-Greiner, Madrid, Spain). Aliquots of 100 μl of the 2× drug dilutions were inoculated into the wells with a multichannel pipette. The microplates were stored at −20°C until used. When the susceptibility test was per-

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TABLE 1. Inoculum size of 24 species of dermatophytes adjusted to 65 to 70% transmission

Species (no. of strains tested)	Inoculum size (CFU/ml)	
	Range	Mean (SD)
E. floccosum (11)	6.0×10^4–2.4×10^6	6.4×10^5 (7.6×10^5)
M. audouinii (8)	2.0×10^4–1.4×10^6	4.9×10^5 (3.9×10^5)
M. canis (11)	1.8×10^4–2.2×10^6	5.8×10^5 (6.8×10^5)
M. cookei (3)	8.0×10^4–1.1×10^6	6.2×10^5 (5.1×10^5)
M. ferrugineum (4)	1.1×10^5–6.0×10^5	3.8×10^5 (2.4×10^5)
M. fulvum (3)	3.0×10^5–8.0×10^5	5.3×10^5 (2.5×10^5)
M. gallinae (3)	2.8×10^5–7.2×10^5	4.7×10^5 (2.2×10^5)
M. gypseum (11)	2.0×10^5–1.2×10^6	6.9×10^5 (3.5×10^5)
M. nanum (3)	7.7×10^5–8.3×10^5	8.0×10^5 (3.0×10^4)
M. praecox (3)	2.0×10^5–5.7×10^5	3.5×10^5 (2.0×10^5
M. racemosum (3)	6.5×10^5–1.5×10^6	9.7×10^5 (4.6×10^5)
T. ajelloi (3)	9.4×10^5–1.9×10^6	1.5×10^6 (4.9×10^5)
T. balcaneum (3)	5.7×10^5–9.4×10^5	7.4×10^5 (1.9×10^5)
T. concentricum (3)	4.4×10^5–6.0×10^5	4.4×10^5 (6.0×10^5)
T. erinacei (7)	1.6×10^4–1.3×10^6	7.3×10^5 (4.2×10^5)
T. interdigitale (11)	2.5×10^5–1.2×10^6	6.6×10^5 (3.3×10^5)
T. mentagrophytes (11)	5.0×10^5–1.7×10^6	9.6×10^5 (3.5×10^5)
T. phaseoliforme (3)	5.0×10^5–1.5×10^6	1.1×10^6 (5.2×10^5)
T. rubrum (11)	2.0×10^5–1.4×10^6	8.8×10^5 (4.4×10^5)
T. schoenleinii (3)	4.0×10^4–2.5×10^5	1.5×10^5 (1.1×10^5)
T. simii (3)	7.5×10^5–1.2×10^6	9.3×10^5 (2.4×10^5)
T. tonsurans (11)	3.0×10^4–9.0×10^5	4.8×10^5 (2.9×10^5)
T. verrucosum (3)	1.5×10^5–2.2×10^5	1.9×10^5 (2.9×10^4)
T. violaceum (3)	7.0×10^4–2.5×10^5	1.8×10^5 (5.7×10^4)

formed, 100 μl of the diluted inoculum suspensions was added to each well to bring the drug dilutions to the final test concentrations. Test concentrations for FCZ ranged from 0.01 to 64 μg/ml, while all remaining agents were tested at 0.0039 to 16 μg/ml. Growth and sterility control wells were included for each isolate tested. The microplate contents were incubated at 28°C, avoiding desiccation of the wells, and were read visually with the aid of a inverted reading mirror after 4 days of incubation. For all azole derivatives, the MIC was the lowest concentration showing prominent growth inhibition (approximately 50% of the growth control). For TF, G-1, and AMB, the MIC was defined as the lowest concentration showing 100% growth inhibition.

Data analysis. Geometric mean MIC, MIC range, the MIC at which 50% of the isolates tested are inhibited (MIC_{50}), and MIC_{90} are provided for all the isolates tested. The significance of differences in mean values was determined by using Student's *t* test with the statistical SPSS package (version 9.0). *P* values of <0.05 were considered statistically significant.

RESULTS

Clearly detectable growth could be visualized for *T. mentagrophytes*, *M. gypseum*, and *T. interdigitale* within 4 days and for all of the remaining isolates within 7 days, with the exception of *T. verrucosum, T. violaceum*, and *T. balcaneum*, which required a full 10 days for detectable growth.

Inoculum count ranges and means are summarized in Table 1. *E. floccosum*, *M. audouinii*, *M. canis*, *M. cookei*, and *T. erinacei* showed the widest ranges (10^4 to 10^6 CFU/ml). *T. ajelloi* and *T. phaseoliforme* showed the highest inoculum concentrations, i.e., 1.5×10^6 and 1.1×10^6 CFU/ml, respectively. The lowest concentrations were those shown by *M. ferrugineum*, *M. praecox*, *T. schoenleinii*, *T. verrucosum*, and *T. violaceum*, which were between 1.5×10^5 and 3.8×10^5 CFU/ml.

The in vitro activities of 10 antifungal agents against 24 species of dermatophytes represented by 508 strains are summarized in Table 2. When all the strains were considered together, the geometric mean MICs of the two novel triazoles UR-9825 and VCZ were apparently the lowest, 0.14 μg/ml, in both cases. However, these results were not significantly different ($P < 0.05$) from those shown by CTZ, 0.17 μg/ml; TF, 0.21 μg/ml; ITZ, 0.22 μg/ml; and MCZ, 0.28 μg/ml. These six antifungal agents were more active ($P < 0.05$) than the rest of the drugs tested. The G-1 and FCZ, geometric mean MICs were highest, i.e., 7.79 and 14.5 μg/ml, respectively. The MIC range for UR-9825 was the narrowest (0.01 to 2 μg/ml), and that for TF was the widest (0.003 to >16 μg/ml). The MIC_{90} of the latter drug was the lowest (0.06 μg/ml). The MIC_{90}s of UR-9825, VCZ, and CTZ were identical (0.25 μg/ml). The MIC_{90}s of FCZ and G-1 were the highest (>16 μg/ml). TF, CTZ, UR-9825, VCZ, and ITZ were the most active drugs against *T. rubrum*; their geometric mean MICs were 0.01, 0.04, 0.09, 0.06, and 0.09 μg/ml, respectively. In contrast, FCZ and G-1 were the least active; their geometric mean MICs were 2.80 and 2.59 μg/ml, respectively. CTZ was highly active against most of the species tested, i.e., *M. canis* (0.04 μg/ml), *T. tonsurans* and *T. violaceum* (0.05 μg/ml), *T. interdigitale* (0.06 μg/ml), *E. floccosum* (0.07 μg/ml), and *T. mentagrophytes* (0.09 μg/ml). TF was very active against all the species; its MICs were as low as 0.003 μg/ml. However, it was ineffective against *M. cookei* and *M. racemosum*. The TF MICs for these two species were > 16 μg/ml. In general these two species were the most resistant ($P < 0.05$). Only UR-9825 and ITZ MICs were low; against *M. cookei* and *M. racemosum*. AMB showed the best activity against *M. fulvum* (0.03 μg/ml); KTZ and TF against *M. ferrugineum* (0.04 μg/ml); FCZ against *M. nanum* (1 μg/ml), *M. racemosum* (1 μg/ml), and *T. tonsurans* (1.91 μg/ml); and G-1 against *T. rubrum* (2.59 μg/ml), *T. tonsurans* (2.42 μg/ml), and *T. verrucosum* (2 μg/ml).

DISCUSSION

A reference method for the antifungal susceptibility testing of dermatophytes is not available. In this study, the NCCLS method for filamentous fungi (11) has been adapted for testing approximately 500 strains of dermatophytes, including the most common species. Modifications to the method included the addition of agents not discussed in the document, a decrease in the incubation temperature to 28°C, and prolongation of the incubation duration from 2 to 3 days to 4 to 10 days. The size of inoculum is considered to be one of the most striking factors in performing antifungal susceptibility testing (4). In this work we have adjusted the inocula to 65 to 70% transmission, as in a previous study (3), where 100 strains of *T. rubrum* were tested under the same conditions. An inoculum density corresponded to 10^4 CFU/ml, which agrees with that recommended by the NCCLS method for filamentous fungi. Here we obtained a concentration range of 4.7×10^3 to 1.5×10^4 CFU/ml for all the species tested. However, it is worth mentioning that with *M. ferrugineum*, *M. praecox*, *T. schoenleinii*, *T. verrucosum*, and *T. violaceum*, the inoculum concentrations were lower. These fungi usually grow slowly and hardly sporulate and may need higher inoculum concentrations than those required by the other species. Interestingly, for *M. racemosum* and *M. cookei*, species characterized by conidia with quite thick walls, the mean MICs of some antifungals were higher than those for the rest of species. Manavathu et al. (8) have demonstrated that the antifungal susceptibility of *Aspergillus* spp., using inocula formed by conidia and hyphae, are

TABLE 2. In vitro activities of 10 antifungal agents against 508 strains of dermatophytes

Species (no. of strains tested)	MIC	Concn (μg/ml)									
		AMB	CTZ	FCZ	G-1	ITZ	KTZ	MCZ	TF	UR-9825	VCZ
E. floccosum (22)	GM[a]	0.11	0.07	4.39	3.48	0.03	0.08	0.06	0.02	0.06	0.03
	Range	0.03–0.5	0.01–0.25	0.5–>64	0.25–>16	0.01–8	0.01–4	0.01–2	0.01–1	0.01–0.5	0.01–8
	MIC_{50}	0.125	0.06	2	4	0.03	0.03	0.03	0.01	0.06	0.03
	MIC_{90}	0.25	0.25	>64	>16	0.125	0.125	1	0.06	0.25	0.125
M. audouinii (8)	GM	0.13	0.04	8.65	15.8	0.04	0.38	0.22	0.02	0.05	0.06
	Range	0.25–0.5	0.01–0.25	0.5–>16	8–>16	0.01–0.125	0.125–1	0.06–2	0.01–0.125	0.03–0.125	0.03–0.06
	MIC_{50}	0.06	0.03	16	8	0.06	0.25	0.125	0.01	0.03	0.03
	MIC_{90}	0.125	0.125	16	>16	0.125	1	2	0.03	0.125	0.125
M. canis (105)	GM	0.29	0.04	5.39	6.05	0.08	0.12	0.06	0.04	0.06	0.04
	Range	0.03–8	0.007–0.5	0.06–>64	0.06–>16	0.01–4	0.01–1	0.01–0.5	0.007–>16	0.01–0.5	0.01–0.5
	MIC_{50}	0.25	0.03	8	>16	0.125	0.25	0.06	0.06	0.06	0.06
	MIC_{90}	1	0.125	16	>16	0.5	0.25	0.25	0.06	0.25	0.125
M. cookei (1)	MIC	>16	>16	4	>16	0.03	8	>16	>16	0.12	0.01
M. ferrugineum (4)	GM	0.14	0.05	4.73	4.73	0.06	0.04	0.06	0.04	0.06	0.05
	Range	0.125–0.25	0.03–0.25	2–16	2–8	0.03–0.125	0.01–0.5	0.06	0.03–0.125	0.03–0.25	0.01–0.5
	MIC_{50}	0.125	0.03	4	4	0.03	0.03	0.06	0.03	0.03	0.03
	MIC_{90}	0.125	0.03	4	8	0.125	0.03	0.06	0.03	0.25	0.06
M. fulvum (1)	MIC	0.03	0.06	16	>16	0.125	2	0.5	0.03	0.25	0.01
M. gallinae (1)	MIC	2	0.01	>16	16	0.125	0.25	0.06	0.01	0.25	0.25
M. gypseum (32)	GM	0.44	0.12	22.37	10.67	0.04	0.23	0.19	0.04	0.13	0.14
	Range	0.06–8	0.03–0.5	8–64	4–16	0.01–0.25	0.03–2	0.06–1	0.03–1	0.01–0.06	0.03–1
	MIC_{50}	0.25	0.125	16	>16	0.03	0.25	0.125	0.06	0.125	0.12
	MIC_{90}	2	0.25	>64	>16	0.12	1	0.5	0.06	0.5	0.5
M. nanum (1)	MIC	0.125	0.03	1	>16	0.03	0.25	0.03	0.06	0.06	0.01
M. praecox (1)	MIC	1	0.01	>16	>16	0.12	1	0.5	0.01	0.25	0.03
M. racemosum (1)	MIC	8	>16	1	>16	0.25	8	16	>16	0.06	1
T. ajelloi (2)	GM	0.35	0.17	2.81	11.31	0.04	0.25	0.12	0.01	0.03	0.02
	Range	0.25–0.5	0.06–0.5	2–4	8–>16	0.03–0.06	0.125–0.5	0.06–0.25	0.007–0.03	0.03	0.007–0.06
T. balcaneum (2)	GM	0.17	0.04	2.8	>16	0.04	0.17	0.25	0.007	0.08	0.06
	Range	0.125–0.25	0.03–0.06	2–4	>16	0.03–0.06	0.06–0.5	0.125–0.5	0.007	0.06–0.25	0.03–0.125
T. concentricum (2)	GM	0.7	0.03	3.98	4	0.01	0.06	0.04	0.007	0.1	0.04
	Range	0.5–1	0.03	2–8	8–>16	0.01	0.06	0.03–0.06	0.007	0.01–1	0.03–0.06
T. erinacei (7)	GM	0.5	0.10	>16	7.19	0.12	0.30	0.15	0.02	0.06	0.09
	Range	0.25–1	0.03–0.25	>16	1–>16	0.03–0.5	0.06–2	0.03–0.5	0.01–0.06	0.03–0.5	0.03–0.25
	MIC_{50}	0.25	0.125	>16	8	0.06	0.25	0.125	0.03	0.06	0.06
	MIC_{90}	0.5	0.25	>16	>16	0.5	2	0.28	0.06	0.5	0.125
T. interdigitale (21)	GM	0.44	0.06	11.88	6.81	0.06	0.38	0.23	0.01	0.10	0.10
	Range	0.25–2	0.01–0.5	0.5–32	0.5–>16	0.01–0.5	0.01–8	0.03–2	0.007–2	0.01–1	0.007–4
	MIC_{50}	0.5	0.06	16	8	0.06	0.5	0.25	0.01	0.125	0.125
	MIC_{90}	0.5	0.25	>64	>16	0.25	1	1	0.06	0.5	0.25
T. mentagrophytes (122)	GM	0.37	0.08	15.08	7.80	0.17	0.46	0.24	0.04	0.09	0.09
	Range	0.125–1	0.01–0.5	0.06–>64	2–>16	0.01–2	0.01–2	0.01–2	0.007–0.5	0.01–0.5	0.01–1
	MIC_{50}	0.5	0.125	16	8	0.25	0.5	0.25	0.06	0.125	0.25
	MIC_{90}	0.5	0.25	>64	>16	1	1	1	0.06	0.25	1
T. phaseoliforme (1)	MIC	0.25	0.125	>16	>16	0.5	2	0.06	0.06	1	1
T. rubrum (144)	GM	0.37	0.04	2.80	2.59	0.09	0.14	0.09	0.01	0.09	0.06
	Range	0.03–>16	0.01–0.5	0.06–>64	0.03–>16	0.01–8	0.01–8	0.01–8	0.003–>16	0.01–2	0.01–1
	MIC_{50}	0.5	0.03	4	4	0.125	0.125	0.125	0.01	0.06	0.06
	MIC_{90}	1	0.125	16	>16	0.5	0.5	0.25	0.06	0.5	0.25
T. schoenleinii (2)	GM	0.25	0.08	>16	8	0.07	0.06	0.04	0.007	0.12	0.02
	Range	0.25	0.06–0.125	>16	8	0.01–0.5	0.03–0.125	0.03–0.06	0.007	0.06–0.25	0.01–0.06
T. simii (2)	GM	0.25	0.08	>16	15.8	0.12	4	1	0.01	0.06	0.06
	Range	0.25	0.03–0.25	>16	8–>16	0.06–0.25	4	0.25–4	0.01–0.03	0.06	0.03–0.125

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TABLE 2—*Continued*

Species (no. of strains tested)	MIC	Concn (μg/ml)									
		AMB	CTZ	FCZ	G-1	ITZ	KTZ	MCZ	TF	UR-9825	VCZ
T. tonsurans (18)	GM	0.16	0.05	1.91	2.42	0.01	0.10	0.13	0.09	0.08	0.04
	Range	0.03–0.5	0.01–0.5	0.06–16	0.125–>16	0.06–0.25	0.01–0.5	0.03–0.5	0.007–0.03	0.03–0.5	0.01–0.25
	MIC_{50}	0.25	0.03	4	2	0.01	0.125	0.25	0.007	0.06	0.04
	MIC_{90}	0.5	0.125	8	>16	0.03	0.25	0.25	0.01	0.5	0.06
T. verrucosum (1)	MIC	0.25	0.25	8	2	0.01	0.5	0.125	0.007	0.03	0.125
T. violaceum (7)	GM	0.18	0.05	2.43	9.6	0.05	0.12	0.08	0.01	0.04	0.04
	Range	0.06–0.25	0.01–0.125	0.5–16	8–16	0.01–0.5	0.03–0.5	0.03–0.25	0.007–0.125	0.01–0.25	0.01–0.125
	MIC_{50}	0.25	0.06	1	8	0.125	0.12	0.06	0.01	0.03	0.03
	MIC_{90}	0.25	0.125	>16	16	0.03	0.25	0.12	0.003	0.125	0.06
All organisms (508)	GM	0.71	0.17	14.5	7.79	0.22	0.5	0.28	0.21	0.14	0.14
	Range	0.03–>16	0.007–>16	0.06–>64	0.03–>16	0.01–8	0.01–8	0.01–>16	0.003–>16	0.01–2	0.007–8
	MIC_{50}	0.25	0.06	8	8	0.125	0.25	0.125	0.03	0.06	0.06
	MIC_{90}	1	0.25	32	>16	0.5	1	0.5	0.06	0.25	0.25

[a] Geometric mean.

very similar, so consequently, the type of inoculum does not have special influence on MICs. However, the widths of conidia and hyphal walls of *Aspergillus* spp. are very similar. Further studies comparing MICs obtained with inocula of conidia and hyphae of the two mentioned species of dermatophytes would be interesting, they could prove if the aforementioned statement is always correct.

In recent years several studies on the in vitro susceptibility of dermatophytes to antifungal drugs have been done and the results have shown considerable variation (2, 6, 10; E. F. Brega, G. Gonzalez, A. W. Fothergill, D. A. Sutton, and M. G. Rinaldi, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 205, 2000.) This variability is probably due to important methodological differences among the laboratories. Norris et al. (12), in an attempt to standardize optimal conditions for dermatophyte susceptibility testing, selected RPMI 1640 medium and 35°C, and 4 days as temperature and time of incubation, respectively, and an inoculum of 10^3 conidia/ml as the most appropiate. Some of these conditions are very different from those recommended for the NCCLS method for filamentous fungi (11). However, the same authors recognized that further studies with more strains and interlaboratory comparisons are needed to validate these factors.

In this study, all the antifungal drugs tested, with the exception of FCZ and G-1, displayed excellent activity, although it is worth mentioning the results shown by the two newest ones, UR-9825 and VCZ. These two potent new triazoles had the lowest geometric mean MICs. UR-9825 had already been very active against pathogenic yeasts and other filamentous fungi (9, 13), even against *Scedosporium prolificans*, an emerging fungus refractory to antifungal treatment with a high occurrence in patients with hematological malignancies (J. Guarro, J. Cano, J. Gené, M. Solé, and A. J. Carrillo-Muñoz, Abstr. 14th Congr. Int. Soc. Hum. Anim. Mycol. p. 84, 2000). The geometric mean MIC of VCZ obtained in this study was similar to that reported by Wildfeuer et al. (17), it was obtained by testing a large number of species of dermatophytes as they did, while using different test conditions, such as a macrobroth dilution method. Both antifungals are promising candidates for clinical trials on the treatment of severe dermatophytoses. Our results also confirmed good in vitro activity of four currently available antifungals, TF, CTZ, MCZ, and ITZ. Similar results have been reported by Hazen (5) under different methodological conditions. He used a macrobroth method and germinated conidia as inoculum. FCZ showed the lowest activity of all the antifungals tested. However, *T. rubrum*, one of the most frequent species causing chronic diseases with frequent remissions and relapses, was more susceptible to fluconazole than were other common species, such as *T. mentagrophytes*, *M. canis*, and *M. gypseum*. Our results agree with those of other authors (16) who demonstrated that susceptibility to FCZ varies greatly among the species.

Overall, our study demonstrated that several antifungal agents are very active against dermatophytes, although these results are clearly species dependent. This, at least in theory, can allow clinicians to adopt different therapeutic options with a high probability of successful results. However, it will be necessary to obtain more clinical data to confirm if this good in vitro efficacy is predictive for clinical outcome.

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