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Letters to the Editor

Comparison of In Vitro Antifungal Susceptibilities of Conidia and Hyphae of Dermatophytes with Thick-Wall Macroconidia

Although the invasive forms of filamentous fungi are generally hyphae, the reference methods (6) recommend the use of conidial suspensions, such as inocula. Some studies testing *Aspergillus* spp. have compared the MICs obtained with conidial suspensions with the MICs obtained with hyphal suspensions by using both forms of inocula, and the MICs were similar (1, 3, 7). This was probably because the thickness of the cell walls of the conidia and hyphae of the fungi tested were similar. However, in some fungi, such as several dermatophytes, the wall of the conidia is considerably thicker than the wall of the hyphae, and we do not know whether the antifungal susceptibilities of these two morphological forms would be different. We have compared how inocula of conidia and hyphae of five species of dermatophytes with thick-wall macroconidia affect the MICs of five antifungal agents.

A total of 14 strains of dermatophytes were tested three times on different days against five antifungal drugs, i.e., clotrimazole (CTZ), itraconazole (ITZ), ravuconazole (RVZ), terbinafine (TF), and voriconazole (VCZ), using both hyphal and conidial inocula (Table 1). Drug dilutions were prepared by the reference methods (6). The final concentrations were 16 to 0.01 μg/ml for all drugs. To obtain an inoculum of macroconidia, the fungus was grown on wheat flour-cow milk agar supplemented with honey at 28°C for 7 to 10 days. The inoculum was prepared by flooding the surface of the agar with distilled water containing 0.05% Tween 80 and scraping the sporulated colonies with the tip of a Pasteur pipette. The suspensions were filtered through sterile gauze to remove the microconidia. The macroconidia retained in the gauze were washed with distilled water and adjusted to a concentration of 5×10^6 macroconidia/ml with a hemacytometer. To obtain an inoculum of hyphae, the fungus was grown on Sabouraud agar at 37°C for 5 to 7 days. Hyphal suspensions were prepared as described previously. They were then vortexed while the pieces of mycelium were broken with a glass rod and filtered through sterile gauze to remove hyphal mats. Hyphal fragments were adjusted to 5×10^6 hyphal fragments/ml. Tests were performed using a broth microdilution method (6). Microdilution trays were incubated at 28 and 37°C (macroconidia and hyphae, respectively) for 4 days. MIC was defined as the lowest drug concentration showing 100% growth inhibition. The significance of the different MICs obtained with the two types of inocula for each antifungal was determined by the Wilcoxon test.

The MICs obtained with conidia were generally higher than those obtained with hyphae for all drugs tested ($P < 0.05$) (Table 1). The highest MICs (≥8 μg/ml) were reached with macroconidial inocula of the following species and drugs: *Microsporum canis* and TF; *Microsporum cookei* and RVZ; *Microsporum gypseum* and CTZ; *Microsporum racemosum* and TF, VCZ, and ITZ; and *Trichophyton ajelloi* and ITZ.

For all species and antifungal drugs, overall agreement (differences not greater than one dilution) between MICs obtained with both types of inocula was low (25.7%). The highest agreement was with *M. canis* (73.4%), and the lowest agreement was with *M. cookei* and *T. ajelloi* (0%).

It is difficult to compare these results with other studies because of the scarcity of data on how the inoculum form affects the MICs for dermatophytes. Nardoni et al. (5) have been the only researchers to have evaluated this. They tested

TABLE 1. In vitro activities of five antifungal agents against 14 isolates of dermatophytes using two types of inocula[a]

Strain[b]	Modal or median MIC (μg/ml)[c]									
	CTZ		ITZ		RVZ		TF		VCZ	
	H[d]	C[d]	H	C	H	C	H	C	H	C
M. canis FMR 6985	2	4	0.03	0.03	4	8	2	8	0.25	1
M. canis FMR 7011	4	4	0.03	0.125	0.5	0.5	8	>16	0.5	1
M. canis FMR 6981	1	2	0.06	0.06	2	4	4	8	0.25	0.5
M. cookei FMR 4408	0.25	1	0.5	4	2	8	2	8	0.5	2
M. cookei IHEM 1294	0.25	4	0.25	2	4	16	0.5	4	0.5	4
M. gypseum FMR 6974	2	8	0.125	2	0.25	4	0.25	1	0.125	0.25
M. gypseum FMR 6968	2	8	0.5	4	0.5	4	0.25	2	0.06	0.125
M. gypseum FMR 6970	2	16	1	4	0.25	2	0.5	2	0.25	0.25
M. racemosum FMR 6313	0.25	2	8	16	0.5	4	2	8	8	>16
M. racemosum IHEM 3452	0.25	1	8	16	0.5	4	2	8	4	8
M. racemosum IHEM 3453	0.25	1	4	8	0.125	2	4	16	4	>16
T. ajelloi FMR 7683	0.25	2	4	16	0.5	2	0.5	2	0.5	2
T. ajelloi IHEM 10479	0.25	4	8	>16	0.125	2	2	8	0.5	2
T. ajelloi IHEM 3757	0.25	2	4	16	0.25	2	0.25	2	1	4

[a] *Paecylomyces variotti* ATCC 36257 and *Aspergillus fumigatus* NCPF 7099 were used as reference strains.
[b] FMR, Faculty of Medicine, University of Rovira and Virgili, Reus, Tarragona, Spain; IHEM, Scientific Institute of Public Health, Louis Pasteur Institute, Brussels, Belgium.
[c] Obtained from three repetitions of each strain. We compared the modal or median MICs obtained with conidial inocula with those obtained with hyphal inocula. When the differences were not greater than one dilution, the MICs were considered to be in agreement.
[d] H and C, hyphal or conidial inoculum, respectively.

TF against *M. canis*, and the MICs obtained with both inocula were similar. The results obtained with other fungal research groups have been contradictory. Nakai et al. (4) tested dimorphic fungi and observed that the MIC of micafungin was higher for the yeast-like form than for the mycelial form. With fluconazole, the results were just the opposite. Other studies (1, 2, 7) indicated that MICs were independent of the form of inoculum. Our results highlight the importance of the type of inoculum and the thickness of the cell walls in determining the in vitro antifungal susceptibility of dermatophytes. Further studies are needed to determine which type of inoculum is more predictive.

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