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In vitro antifungal susceptibility of *Alternaria* spp. and *Ulocladium* spp.

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Sir,

Isolates of the fungal genus *Alternaria* are usually found as soil saprophytes and plant pathogens, although some species can play an important role in causing disease in both healthy and immunocompromised patients. The most common clinical manifestations associated with *Alternaria* spp. infections include keratitis, peritonitis, osteomyelitis, pulmonary and cutaneous infections, and subcutaneous infections in the case of *Ulocladium*,[1] a morphologically closely related genus. There are scarce data about the *in vitro* antifungal activity against these fungi, probably because of the lack of reference methods for *in vitro* antifungal susceptibility testing of dematiaceous fungi.

In this study, the *in vitro* antifungal activity of amphotericin B, flucytosine, fluconazole, itraconazole, ketoconazole and miconazole was evaluated against 20 isolates of *Alternaria* spp. (four *Alternaria alternata*, two *Alternaria chlamydospora*, two *Alternaria dianthicola*, one *Alternaria geophila*, two *Alternaria infectoria*, three *Alternaria longipes* and six *Alternaria tenuissima*) and seven *Ulocladium* spp. (four *Ulocladium chartarum* and three *Ulocladium botrytis*) from very different sources. The antifungal agents were provided by the manufacturer in standard powder form, with the exception of amphotericin B and fluconazole, which were commercial intravenous preparations (Fungizone and Diflucan, respectively). *Paecilomyces variotti* ATCC 36257 was included as the control.

MICs of antifungal agents were obtained by the broth microdilution method according to the recommendations of the NCCLS (M38-P)[2] with some modifications. Conidial inoculum suspension was standardized by the haemocytometer method and it was tested at a final concentration of $1–5 \times 10^4$ conidia/mL. The final test drug dilutions were 0.12 to 64 mg/L of fluconazole, 0.25 to 128 mg/L of flucytosine, and 0.03 to 16 mg/L of amphotericin B, ketoconazole, miconazole and itraconazole. Incubation was at 30°C for 48 h. Flucytosine and azole MICs were defined as the lowest drug dilution that resulted in slight turbidity (approximately $\leqslant$ 25%) in comparison with the control growth. The MIC of amphotericin B was defined as the lowest drug dilution that inhibited fungal growth completely.

Table. Antifungal susceptibility of *Alternaria* and *Ulocladium* spp.

Organism	No. strains	Antifungal agents	MIC (mg/L) range	MIC_{50}	MIC_{90}	geometric mean
Alternaria spp.	20	amphotericin B	0.12–>16	1	2	1.27
		miconazole	0.5–8	4	4	3.75
		itraconazole	0.12–2	0.5	1	0.46
		fluconazole	16–>64	32	>64	49.54
		ketoconazole	0.5–8	2	2	1.67
		flucytosine	>128	>128	>128	251.18
Ulocladium spp.	7	amphotericin B	1–>16	2	>16	2.68
		miconazole	0.5–16	4	16	2.96
		itraconazole	0.06–>16	16	>16	4.81
		fluconazole	8–>64	>64	>64	69.63
		ketoconazole	0.12–>16	8	>16	4.39
		flucytosine	>128	>128	>128	251.18

The results of the antifungal susceptibility testing are summarized in the Table. For purposes of comparison, we have considered the breakpoints for defining resistance published by Sutton *et al.*[3] (⩾2 mg/L of amphotericin B, ⩾1 mg/L of itraconazole, ⩾16 mg/L of miconazole and ketoconazole, ⩾64 mg/L of fluconazole and ⩾32 mg/L of flucytosine). According to these criteria, all isolates of *Alternaria* spp. were susceptible to miconazole and ketoconazole, 20% of strains were resistant to itraconazole and 45% of strains were resistant to amphotericin B and fluconazole. All isolates were resistant to flucytosine. In contrast, *Ulocladium* spp. showed a higher degree of resistance to all antifungals: 14.3% of strains were resistant to miconazole, 28.6% to ketoconazole, 71.4% to itraconazole and 85.7% to amphotericin B and fluconazole. All isolates were resistant to flucytosine.

In the last 10 years more than 50 cases of *Alternaria* spp. infections have been published, although only in a few of these have *in vitro* antifungal susceptibility data been reported.[4–6] In general, it seems that *Alternaria* spp. are resistant to flucytosine and the activity of amphotericin B, ketoconazole and miconazole is variable. High *in vitro* activity of itraconazole was reported. This agrees in part with our results, although in our case ketoconazole and miconazole also showed high efficacy.

Our results also agree with those reported from the clinical setting, particularly in the case of itraconazole, i.e. in 11 out of 12 clinical cases treated with this drug the outcome was positive. The clinical efficacy of ketoconazole and amphotericin B has been more variable. Six out of 12 cases were resolved with ketoconazole and eight out of 16 with amphotericin B. Treatment with flucytosine was always in combination with amphotericin B and successful results were obtained in four cases out of six. In contrast to our results, treatment with fluconazole brought a positive outcome in all five clinical cases in which it was used. Only one successful case of treatment with miconazole has been reported, which was in combination with fluconazole. In some cases alternariosis was resolved by combining antifungal drugs with surgical resection, whereas in others only by surgical resection or by stopping the corticosteroid therapy.

In conclusion, our *in vitro* results generally agree with the clinical data reported in the literature and confirm itraconazole as a promising drug for the treatment of *Alternaria* spp. infections. Voriconazole, the new triazole derivative, has also demonstrated good *in vitro* efficacy against *A. alternata*[7] but its possible clinical application should be established by clinical use.

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