ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2005, p. 3495–3497 Vol. 49, No. 8
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.8.3495–3497.2005

Antifungal Susceptibility of 596 *Aspergillus fumigatus* Strains Isolated from Outdoor Air, Hospital Air, and Clinical Samples: Analysis by Site of Isolation

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Received 4 April 2005/Returned for modification 30 April 2005/Accepted 19 May 2005

We analyzed the activities of six antifungal drugs (amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin, and micafungin) against 596 *Aspergillus fumigatus* strains isolated from outdoor air, hospital air, and clinical samples. We did not find differences among the susceptibilities by site of isolation.

During the last few years, there has been an increase in the number of cases of invasive aspergillosis (8), a disease with a very high mortality. Infecting strains may be acquired both inside and outside the hospital.

Voriconazole has proven to be superior to amphotericin B and is now the drug of choice for the primary treatment of invasive aspergillosis (9, 13).

Despite the lack of definitive data correlating in vitro susceptibility results with clinical response, in vitro antifungal activity against *Aspergillus fumigatus* should be considered a sine qua non when selecting therapy.

We are not aware of large studies comparing the antifungal susceptibility of *A. fumigatus* isolated from different sources. We analyzed the activity of six antifungal drugs against 596 *A. fumigatus* strains isolated from three different sites and at three different times: 175 were from outdoor air at selected points across the province of Madrid (August 2002 to May 2003), 135 were from hospital air as part of our environmental filamentous fungi surveillance (1994–2003), and 286 were from clinical samples of hospitalized patients (1999–2003).

The air samples were collected by use of the Merck Air Sampler MAS 100 with a final air volume of 200 liters per plate. Every sample was cultured on both media used, Sabouraud dextrose and Czapek agars (pair of samples). All strains were cultured in Sabouraud dextrose agar and identified by conventional methods. The clinical strains belonged to 182 patients (33 patients had proven or probable invasive aspergillosis, whereas 149 patients were colonized). No patients had allergic disorders.

The antifungal drugs used were amphotericin B (Sigma Chemical Co., St. Louis, Mo.), itraconazole (Janssen Pharmaceutical Research and Development, Madrid, Spain), voriconazole (Pfizer Pharmaceutical Group, New York, N.Y.), posaconazole (Schering-Plough Research Institute, Kenilworth, N.J.), caspofungin (Merck Research Laboratories, Rahway, N.J.) and micafungin (Fujisawa GmbH). The broth microdilution method was performed according to CLSI guidelines (14). The final concentration of the drugs in the wells ranged from 0.007 to 8 μg/ml (10 twofold dilutions) for echinocandins and from 0.03 to 16 μg/ml for the remaining antifungal drugs. The trays were incubated at 35°C and read at 48 h. The MIC endpoint for the azoles and amphotericin B was defined as the lowest concentration that produced complete inhibition of growth, whereas the minimum effective concentration (MEC) endpoint for caspofungin was defined according to published methods (1, 11). Quality control was ensured by testing the following strains: *Aspergillus flavus* ATCC 204304 and *A. fumigatus* ATCC 204305. All results were within the recommended limits of the CLSI.

The log MICs for each antifungal and origin were compared by using the Student *t* test. The alpha value was set at 0.05, and all *P* values were two-tailed.

For all 596 strains, the in vitro activity of each antifungal drug, expressed as the geometric mean of the MICs, MIC_{90}, MIC_{50}, and range (μg/ml), is shown in Table 1. Voriconazole was the most active drug, followed by posaconazole, itraconazole, and amphotericin B. The interpretation of echinocandins (caspofungin and micafungin) was different and showed very low MECs that were always <0.007 μg/ml, irrespective of the origin of the isolates. However, in the absence of a cutoff, the significance of these values remains unknown. Caspofungin and micafungin were equivalent in our study. Nine (1.5%) strains presented an MIC of ≥ 4 μg/ml to amphotericin B: two were from hospital air, one was from outdoor air, and six were from clinical samples. All strains presented an MIC of ≤ 2 μg/ml for the azole derivatives.

There were no significant differences between the susceptibilities of *A. fumigatus* isolates by site of isolation. We did not find differences between the strains isolated from infected patients and those isolated from colonized patients or in the source of the clinical strain.

To our knowledge, our susceptibility study includes the largest number of *A. fumigatus* strains tested in a single series (2–4, 6, 10, 15). We were not able to find significant differences in antifungal susceptibilities between strains obtained from the environment or from patients.

In our study, we only found nine strains (1.5%) that showed an MIC of >2 μg/ml for amphotericin B. Six of these were collected from clinical samples. *A. fumigatus* strains with MICs

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TABLE 1. In vitro activity of each antifungal drug against *A. fumigatus* analyzed by source of isolation

Source of isolation (no. of strains)	Antifungal drug	MIC or MEC (μg/ml)		MIC or MEC geometric mean (μg/ml)	MIC or MEC range (μg/ml)
		50%	90%		
Total (596)	Amphotericin B	2	2	2.017	1–4
	Itraconazole	1	1	0.810	0.125–2
	Voriconazole	0.25	0.5	0.303	0.125–2
	Posaconazole	0.5	0.5	0.385	0.125–1
	Caspofungin	<0.007[a]	<0.007		0.007
	Micafungin	<0.007	<0.007		0.007
Outdoor air (175)	Amphotericin B	2	2	2	1–4
	Itraconazole	1	1	0.798	0.5–1
	Voriconazole	0.25	0.5	0.319	0.125–2
	Posaconazole	0.5	0.5	0.364	0.125–0.5
	Caspofungin	<0.007	<0.007		0.007
	Micafungin	<0.007	<0.007		0.007
Hospital air (135)	Amphotericin B	2	2	2.010	1–4
	Itraconazole	1	1	0.831	0.125–1
	Voriconazole	0.25	0.5	0.295	0.125–1
	Posaconazole	0.5	0.5	0.419	0.125
	Caspofungin	<0.007	<0.007		0.007
	Micafungin	<0.007	<0.007		0.007
Clinical samples (286)	Amphotericin B	2	2	2.019	1–4
	Itraconazole	1	1	0.808	0.25–2
	Voriconazole	0.25	0.5	0.297	0.125–2
	Posaconazole	0.5	0.5	0.383	0.125–1
	Caspofungin	<0.007	<0.007		0.007
	Micafungin	<0.007	<0.007		0.007

[a] The effect of the echinocandins appeared in the lowest concentration studied (0.007 μg/ml); therefore, it was not possible to obtain the exact MEC without studying lower dilutions.

above 2 μg/ml for amphotericin B are associated with a high probability of therapeutic failure (12), although there are no studies that have determined a reliable cutoff to define a strain as resistant or susceptible.

Resistance to the newer triazoles is very rare (5, 7), and we did not find any strains which were resistant to any of the azoles tested.

Caspofungin and micafungin exhibited powerful antifungal activities, but the absence of a standardized endpoint does not allow us to compare their activities with the other molecules. It is also important that we considered as MIC endpoints for azole derivatives and amphotericin B those antifungal dilutions that produced complete inhibition of growth, whereas the MEC endpoint for caspofungin was the antifungal dilution that produced a change in the growth of the fungus but no inhibition in the growth. This could explain the differences in activity between the echinocandins and the other molecules studied, an activity that may be not correlated with the in vivo response.

One shortcoming of our study is that a large number of our strains were collected from patients who were not taking antifungal therapy. Despite the wide use of antifungal agents among clinical patients, we were not able to demonstrate significant differences in antifungal susceptibilities of *A. fumigatus* isolates obtained from patients or from the environment.

This study was partially presented at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C. (abstract 1016).

This study was partially financed by research funds from the Comunidad de Madrid (CAM Ref. 08.2/0026/2001 1) and by grant number 02/0016-1 from Fondos de Investigación Sanitaria and from Red Española de Investigación en Patología Infecciosa C/03/14 (REIPI). Jesús Guinea received a predoctoral grant from the Universidad Complutense, Madrid, Spain.

We thank Thomas O'Boyle for his help in the translation of the article.

This study does not present any conflict of interest for any of its authors.

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