

Surveillance of Azole Resistance among Environmental Isolates of *Aspergillus fumigatus* Species Complex in a General Hospital in Madrid

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Introduction

- Recent studies have reported the recovery of triazole-resistant *Aspergillus fumigatus* isolates from hospital indoor air, and suggested that patients with invasive aspergillosis (IA) due to azole-resistant strains might acquire the fungus from the environment.
- A. lentulus* and other close relatives of *A. fumigatus* have been implicated in several cases of IA attended in Spanish medical centers [1-3], and have also been found in outdoor hospital air [4].
- In order to determine triazole resistance, we retrospectively screened a large collection of *A.fumigatus* species complex (AFSC) isolates recovered from air samples collected in a Spanish Teaching Hospital over a 12-year period (2000-2011).
- All studied isolates were identified to the species level to assess the prevalence of cryptic species in the hospital indoor environment.

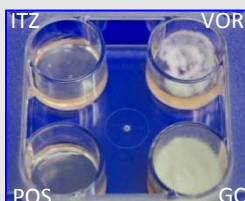
Materials and Methods

We identified 322 AFSC isolates to the species level by combining classical and molecular methods and screened them for possible triazole resistance by culture on agar media containing itraconazole (ITZ, 4 µg/mL), posaconazole (POS, 0.5 µg/mL), and voriconazole (VOR, 1 µg/mL). A growth control (GC, medium without antifungal) was included in each screening plate.

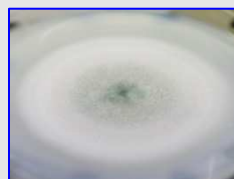
We also determined MICs to amphotericin B (AMB) and triazoles according to the CLSI guidelines for azole screening positive *A. fumigatus* 'sensu stricto' isolates and all isolates belonging to cryptic species.



Neosartorya udagawae



Screening of azole resistance



Aspergillus lentulus

References

- Alhambra A *et al.* 2008. Rev Iberoam Micol,25:246-249.
- Escribano P *et al.* 2013. Antimicrob Agents Chemother 57: 2815-2820.
- Peláez *et al.* 2013. J Med Microbiol 62: 474-478.
- Mortensen *et al.* 2010. Antimicrob Agent chemother 54:4545-4549.

Results

- We identified 312 (96.9%) isolates as *A. fumigatus* 'sensu stricto' and 10 (3.1%) as cryptic species (5 *Neosartorya hiratsukae*; 2 *N. udagawae*; and 3 *A. lentulus*).
- Cryptic species came from air samples taken at surgical (10%), protected (40%) and non-protected (50%) areas. The first isolation dated back from 2007.
- Thirty *A. fumigatus* 'sensu stricto' isolates grew on triazole screening test : 18 were ITZ+, 7 VOR+, 4 ITZ+ VOR+, and 1 ITZ+ POS+. MIC values for these isolates are shown in Table 1.
- A. lentulus* and *N. udagawae* exhibited higher MICs to azoles than *A. fumigatus* 'sensu stricto'.

Table 1. Geometric mean (µg/ml) and range of MICs for selected antifungal agents against the environmental *Aspergillus fumigatus* 'sensu stricto' isolates with screening positive test and isolates belonging to cryptic species.

Fungal Species	AMB	ITZ	VOR	POS
<i>A. fumigatus</i> 'sensu stricto' (30)	0.945 (0.5-2)	0.603 (0.25-2)	0.430 (0.25-1)	0.215 (0.125-0.5)
<i>Neosartorya hiratsukae</i> (5)	0.659 (0.5-1)	0.164 (0.125-0.5)	0.217 (0.25-1)	0.092 (0.06-0.25)
<i>Neosartorya udagawae</i> (2)	2 (2)	0.707 (0.5-1)	0.353 (0.25-0.5)	1 (1)
<i>Aspergillus lentulus</i> (3)	2 (2)	2.519 (1-8)	2 (2)	0.396 (0.25-0.5)

Conclusions

- To our knowledge, this is the first study describing the presence of cryptic *Aspergillus* section *Fumigati* species in the hospital indoor environment in Spain.
- The results of this study suggest that triazole resistance among environmental *A. fumigatus* 'sensu stricto' isolates is not a cause for concern in our institution. However, further environmental surveillance of cryptic members of *A. fumigatus* species complex is recommended to detect any possible emergence of azole-resistant strains.