Aspergillus section *Fumigati* – Epidemiological trends. A perspective from a National Reference Laboratory.

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Objectives

Aspergillus species have emerged as an important cause of life-threatening infections in immunocompromised patients. *Aspergillus fumigatus* is the most frequent agent of aspergilosis and reports on infections caused by this species or its siblings are becoming more frequent, together with the increasing number of at risk patients. Nowadays, due to the rising concerns on emerging antifungal resistance, the epidemiological surveillance for clinical and environmental isolates is mandatory.

The overall objective of the project is to understand the epidemiology of the *Aspergillus* isolates (species and antifungal resistance) collected in the Portuguese National Reference Laboratory through our surveillance system on *Aspergillus*.

Methods

During the period 2013-2017, 103 *Aspergillus* section *Fumigati* isolates were collected at the National Health Reference Dr. Ricardo Jorge, through the surveillance system on *Aspergillus.* Isolates were obtained from different patient samples and from 15 healthcare institutions of all country. All isolates were plated for growth as single colonies on malt extract agar with chloramphenicol. These isolates were identified on the basis of macro and microscopic morphology and through the use of molecular tools. Genomic DNA was prepared from each isolate and the sequencing of the Internal Transcribed Spacers (ITS) regions as well as of the gene codifying to calmodulin.

Surveillance of azole resistance was performed firstly using Sabouraud dextrose agar supplemented with itraconazole (ICZ), voriconazole (VCZ), and posaconazole (PCZ). When growth was observed, the minimal inhibitory concentration (MIC) was determined by broth microdilution method. In case of doubt, a specific PCR for detection of mutations in

the *Cyp51A* gene of *A. fumigatus* was performed using the AsperGenius® multiplex real-time PCR assay.

Results

From the isolates collected during the study period, 94 were from clinical (human) sources, 2 from animals diagnosed with aspergillosis and 7 from environmental sources (occupational settings) obtained by air or surfaces samples

Isolates were obtained from 90 patients (53 males, 34 females, 3 not known), with ages ranging from 37 days to 88 years old. Most of the isolates (98%) were from respiratory specimens. The underlying diseases reported are, among others, cystic fibrosis, COPD, HIV, asthma, and neoplasms. In total, 98 *A. fumigatus* sensu stricto isolates were identified, followed by 3 *A. lentulus* and 2 *A. felis*. In 7 cases, the morphological identification did not matched with the correct species. Interestingly, the 5 cryptic species were from the same hospital. Regarding susceptibility, relevant and residual growths were obtained in azole resistance screening media (Table 1). The positive results were then screened by microdilutions and the detection of Cyp51A mutations and possible resistances were not confirmed.

Conclusions

The understanding of local resistance patterns is valuable to assess shifts in the epidemiology of *Aspergillus* (and therefore, to manage therapeutic approaches). In our collection of *Fumigati* isolates, 5% of them were cryptic species. Although we did not confirm azole resistance by microdilution or detection of Cyp51A mutations, the MIC values obtained suggest that the median values are higher than what is described in other studies (1.3 to ICZ, 0.77 to PCZ), which may explain the growth in the screening media and may suggest a local epidemiology.

Table 1. Results of inoculation of the studied *Fumigati* isolates in Sabouraud dextrose agar supplemented with itraconazole (ICZ), voriconazole (VCZ), and posaconazole (PCZ).

	ICZ			VCZ			PCZ		
Isolate growth in culture	Relevant	Residual	Negative	Relevant	Residual	Negative	Relevant	Residual	Negative
A. fumigatus sensu stricto (N=91*)	13	17	61	0	1	90	3	3	85
A. lentulus (N=3)	2	0	1	1	1	1	2	0	1
A. felis (N=1*)	1	0	0	1	0	0	2	0	0

*The remaining isolates lost their viability and did not grow in the positive control