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Antifungal Resistance in *Aspergillus fumigatus*: Environmental Conditions & Clinical Implications

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Context

Air is the most common medium of fungal spore dispersal and is the main route of infection for respiratory fungal infections [1].
Increasing levels of antifungal resistance have been reported in several European countries. In 2022 the WHO launched the Fungal Priority Pathogens List in which ***A. fumigatus* was included in the critical group** partly due to its emergence of environment-related resistance [2].

AIM

Analyze the presence of filamentous fungi of clinical interest in ambient air samples.

- Study of the annual seasonal variation and influence of environmental factors.
- Characterization and distribution of fungal species isolated in ambient air.

Analyze and characterize antifungal susceptibility in environmental isolates of *A. fumigatus*.

- Characterization of antifungal susceptibility to azoles, echinocandins and amphotericin B.
- Analyze the presence of Cyp51A mutations in resistant isolates and study the relationship between isolates.

Methods

A comprehensive monitoring of fungal presence in the air was conducted at two locations (urban and semiurban environment) in Madrid-Spain.

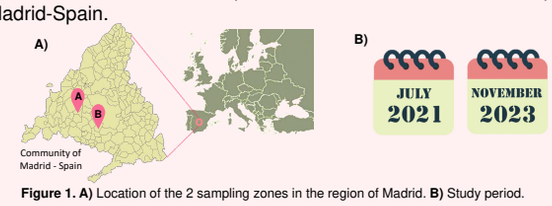

Figure 1. A) Location of the 2 sampling zones in the region of Madrid. **B)** Study period.

Ambient air total suspended particles were collected monthly by filtering air according to UNE CEN/TS 16115-1:2013 Technical Specification [3]. During each sampling campaign, ambient air temperature (°C), relative humidity (%) and atmospheric pressure (hPa) data were collected minutely by means of a calibrated sensor in the sampler and subsequently averaged.

Each isolate was grown, subcultured and confirmed to species level by sequencing the ITS1-5.8S-ITS2 regions and *benA* gene [4].

Figure 2. Framework of the environmental sampling process.

A. fumigatus was screened for azole resistance using EUCAST E.Def 10.1 [5] and confirmed by EUCAST E.Def 9.4. [6] Resistance mechanisms were studied by sequencing the gene *cyp51A* including its promoter [1].

All resistant and a portion of the susceptible *A. fumigatus* isolates were typed by TRESPERG method [7].

Table 1. Panel of 4 genes used for characterization using the TRESPERG typing method.

Afu3g08990	*cspA* for cell surface protein A
Afu2g05150	MP2 antigenic galactomannan protein
Afu6g14090	Hypothetical protein with CFEM domain
Afu1g07140	*erg*4B formed by 12-mer repeats

Results

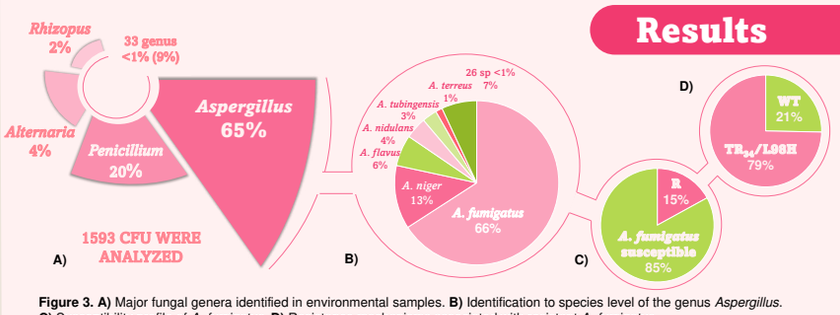

Figure 3. A) Major fungal genera identified in environmental samples. **B)** Identification to species level of the genus *Aspergillus*. **C)** Susceptibility profile of *A. fumigatus*. **D)** Resistance mechanisms associated with resistant *A. fumigatus*.

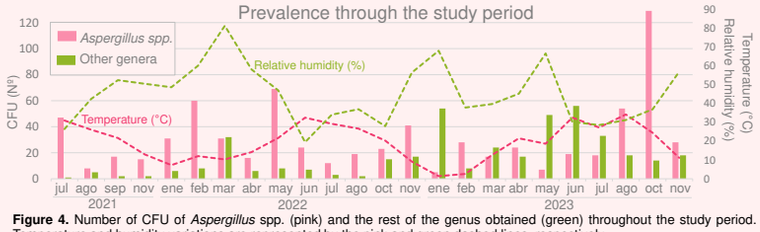

Figure 4. Number of CFU of *Aspergillus* spp. (pink) and the rest of the genus obtained (green) throughout the study period. Temperature and humidity variations are represented by the pink and green dashed lines, respectively.

The study of annual seasonal variation and the influence of environmental factors did not yield significant results relevant to the prevalence of filamentous fungi in environmental air.

	Nº sp	Nº genus	CFU	*Aspergillus spp*	*A. fumigatus*	*R-A.fumigatus*	*Alternaria spp.*	*Penicillium spp.*	*Rhizopus spp.*
Nº sp		0,79	0,52*	0,02	-0,03	-0,16	0,42*	0,67	0,14
Nº genus	0,79		0,52*	-0,06	-0,07	-0,22	0,60*	0,72	0,05
CFU	0,52*	0,52*		0,65	0,52	0,06	0,30*	0,59*	0,15
Aspergillus spp.	0,02	-0,06	0,65		0,73	0,15	-0,22	-0,05	0,16
A. fumigatus	-0,03	-0,07	0,52	0,73		0,33*	-0,18	-0,06	-0,09
R-A. fumigatus	-0,16	-0,22	0,06	0,15	0,33*		-0,04	-0,19	-0,20
Alternaria spp.	0,42*	0,60*	0,30*	-0,22	-0,18	-0,04		0,41*	-0,20
Penicillium spp.	0,67	0,72	0,59*	-0,05	-0,06	-0,19	0,41*		0,02
Rhizopus spp.	0,14	0,05	0,15	0,16	-0,09	-0,20	-0,20	0,02	
Temperature	0,01	0,02	-0,02	0,05	-0,23	0,14	0,09	-0,14	0,12
Relative humidity	0,01	-0,08	0,06	-0,14	0,10	-0,06	0,14	0,16	-0,21
Atmospheric pressure	0,10	-0,03	0,08	0,02	0,04	0,07	-0,10	0,16	0,14

1 / 0.5 / -0.5 / -1

Figure 5. Correlation between environmental factors and the presence of fungal species in ambient air samples. (* $p < 0.05$). Colour intensity according to Spearman's correlation strength green if positive and pink negative.

GENOTYPES

A total of 133 *A. fumigatus* were genotyped, with 43 TRESPERG types detected. Among the 76 resistant ones, 10 types were identified.

Table 2. TRESPERG genotypes of resistant strains and associated resistance mechanisms.

TRESPERG type (Nº isolates)	*cyp51A*	*cyp51B*	*hmg1*
t02m1.1c09e05 (55)	TR_{34}/L98H	-	-
t02m1.1c09e16 (2)	TR_{34}/L98H	-	-
t02m1.1c09e22 (1)	TR_{34}/L98H	-	-
t03m1.1c05Ae07 (6)	WT	WT	WT
t03m1.1c08Ae08 (3)	WT	WT	WT
t03m1.1c10e06 (2)	WT	WT	WT
t04Am1.3c08Be07 (1)	WT	WT	WT
t04Bm1.2c08Ae07 (1)	TR_{34}/L98H	-	-
t04Bm1.2c20e06 (1)	TR_{34}/L98H	-	-
t09m1.1c04e13 (4)	WT	WT	WT

Environmental TRESPERG genotypes have also been identified in the clinical samples.

Table 3. TRESPERG genotypes identified in both environmental (E) and clinical (C) background, and its susceptibility profile (susceptible framed in blue and resistant in red).

	E	C
t02m1.1c09e05	55	2 / 1
t02m6.1c08Ae11	1	1
t03m1.1c05Ae07	6	4
t03m1.1c05Ae09	1	7
t03m1.1c08Ae07	2	1 / 13
t03m1.1c08Ae08	3 / 1	1
t03m1.1c08Ae09	1	2
t03m1.1c09e07	1	2
t03m1.3c08Ae07	2	1
t03m1.3c09e02	1	3
t04Am1.3c08Ae07	1	1
t04Am1.3c08Be07	1 / 5	1
t04Am3.4c17e11	1	1
t26m1.1c08Be07	2	7

CONCLUSIONS

1. There were no observed seasonal variations and environmental factors such as humidity, temperature, and atmospheric pressure did not significantly impact the presence of filamentous fungi in the air.
2. Azole resistant *A. fumigatus* strains were found in air samples from two different locations in Madrid.
3. The TR_{34}/L98H mutation in the *cyp51A* gene was the sole mechanism associated with the observed azole resistance found in *cyp51A*.
4. Resistant strains without mutations associated with the analyzed gene were also isolated, comprising 21% of the total resistant strains.
5. The presence of resistant *A. fumigatus* strains in the environment underscores the importance of monitoring to understand how resistance develops and spreads.
6. A greater diversity of genotypes was observed among susceptible *A. fumigatus* strains compared to the resistant ones.
7. The prevalence of specific resistant genotypes in the environment, which closely resemble clinical isolates, should be explored in depth for a better understanding of the risks and transmission routes. Furthermore, it emphasizes the need for implementing surveillance programs, control strategies, and assessment of clinical implications.

SHARING IS ENCOURAGED