Prevalence of fungal colonization in cystic fibrosis a retrospective study over 3 years in Buenos Aires, Argentina

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Purpose

In cystic fibrosis patients (CF), fungal colonization of the respiratory tract is frequently found. However, the knowledge on prevalence rates of molds and yeasts in patients with this genetic disorder in Argentina is scarce. The aim of the present work is to give an overview of the diversity and epidemiology of fungal species in CF patients in our country.

Methods and Materials

Over a 3-year period, a total of 132 fungal strains isolated from sputa of 38 CF patients from Argentina were investigated. Isolates were identified according to standard macroscopic and microscopic morphological criteria. In order to identify mold isolates to subspecies level and uncover cryptic species, sequence analysis of the fungal ITS regions of the ribosomal DNA (primers ITS1/ITS4), the beta-tubulin region (primers Bt2a/Bt2b) and the calmodulin region (CMD5/CMD6) were performed. For species identification of clinically relevant *Scedosporium*, a multiplex PCR was also carried out as described by Harun et al., 2011.

Results

As it is depicted in Figure 1, about 73.7% of CF patients were colonized by *Aspergillus s.* section *Fumigati,* 21.1% by *Aspergillus* section *Flavi,* 18.4% by *Aspergillus* section *Terri*, 15.8 % by *Penicillium/Talaromyces sp*., 10.5% by *Aspergillus* section *Nigri* and *Exophiala sp*., 7.9% by *Scedosporium sp*., 2.6% by *Aspergillus* section *Usti* and 15.8% by other *fungi* including *Alternaria sp*., *Paecilomyces sp*. and *Trichosporon sp*. The occurrence of rare fungi in CF patients like *Ramsamsonia argillacea*, *Cephalotheca foveolata*, *Phoma haematocycla* and *Rhodothorula sp*. was sporadically observed. In some cases, fungal coinfections were detected (e.g. *Aspergillus fumigatus* and *Exophiala dermatitidis*; *Aspergillus terreus/A. fumigatus* and *Scedosporium aurantiacum*).

Among the genera *Aspergillus*, *A. fumigatus* was the most prevalent species which accounted for 53.6% of isolates, followed by *Aspergillus flavus* and A. *terreus* with 14.3% of isolates each, *Aspergillus niger* accounted for 8.3%, Aspergillus *calidoustus* for 3.6%, *Aspergillus alabamensis* for 2.4%, and *Aspergillus pseudoterreus*, Aspergillus *lentulus* and Aspergillus *parasiticus* for 1.2% each (Figure 2). Among the genera *Scedosporium*, the most common species were *Scedosporium apiospermum* (6.1% of isolates) and *S. aurantiacum (3.8% of isolates)* (Figure 1) and these results correlated well with those from the multiplex-PCR method described by Harun et al., 2011 (Figure 3). All molecular identifications were successfully correlated with macro and micro morphology analysis (Figure 4).

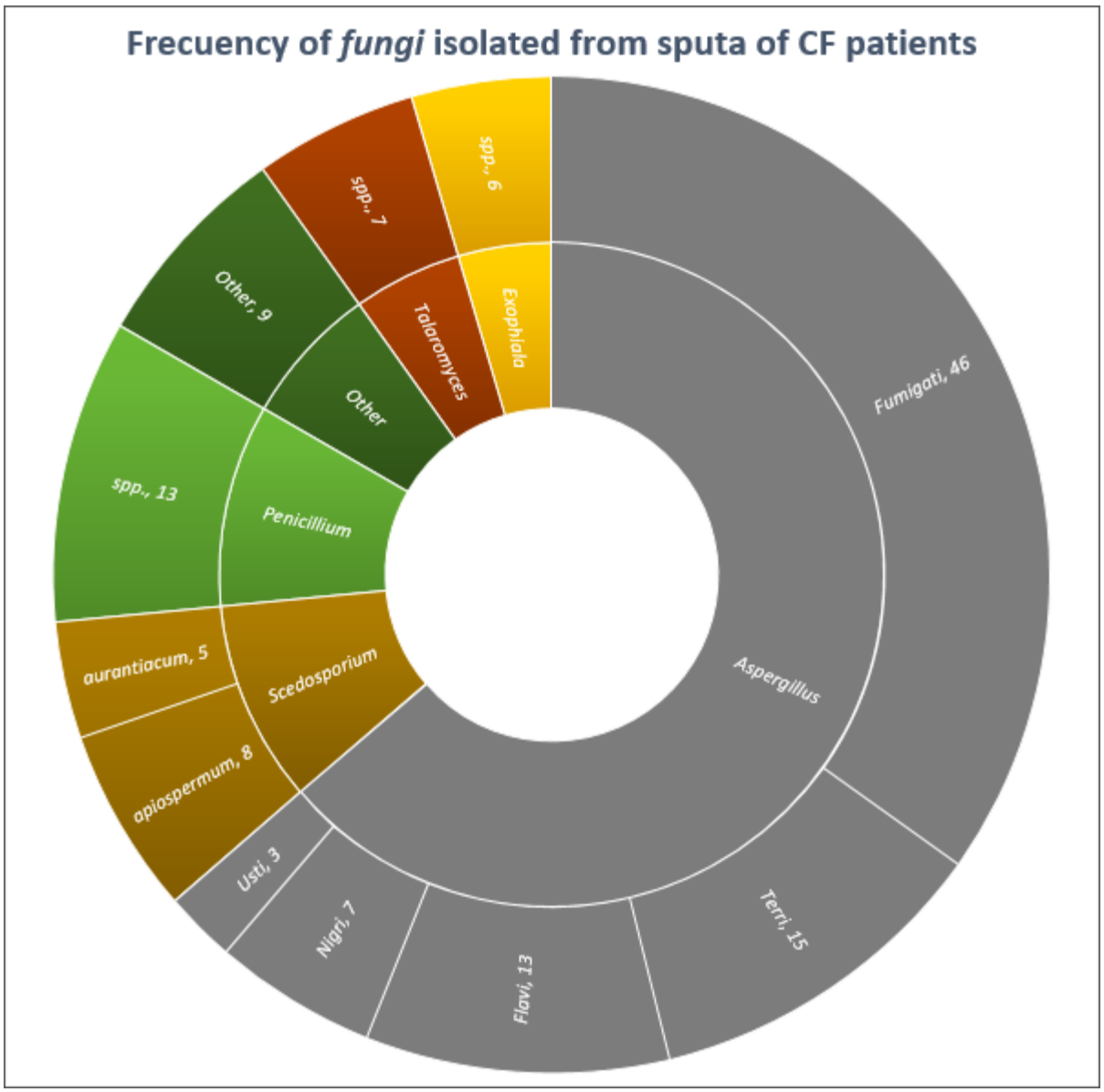

Figure 1 – Frequency of fungi isolated from sputa of CF patients. The group "Other" includes isolates of *Alternaria sp*., *Paecilomyces sp*., *Trichosporon sp., R. argillacea, C. foveolata, P. haematocycla* and *Rhodothorula spp*. Species were identified by nucleotide sequencing and phylogenetic analysis of ITS, beta-tubulin and Calmodulin genes. In some cases, it was not possible to identify isolates to species level (e.g. some *Penicillium/Talaromyces* and *A. flavus/A. oryzae*). In such cases, isolates were annotated as *Penicillium sp.* or *Aspergillus* section *Flavi*.

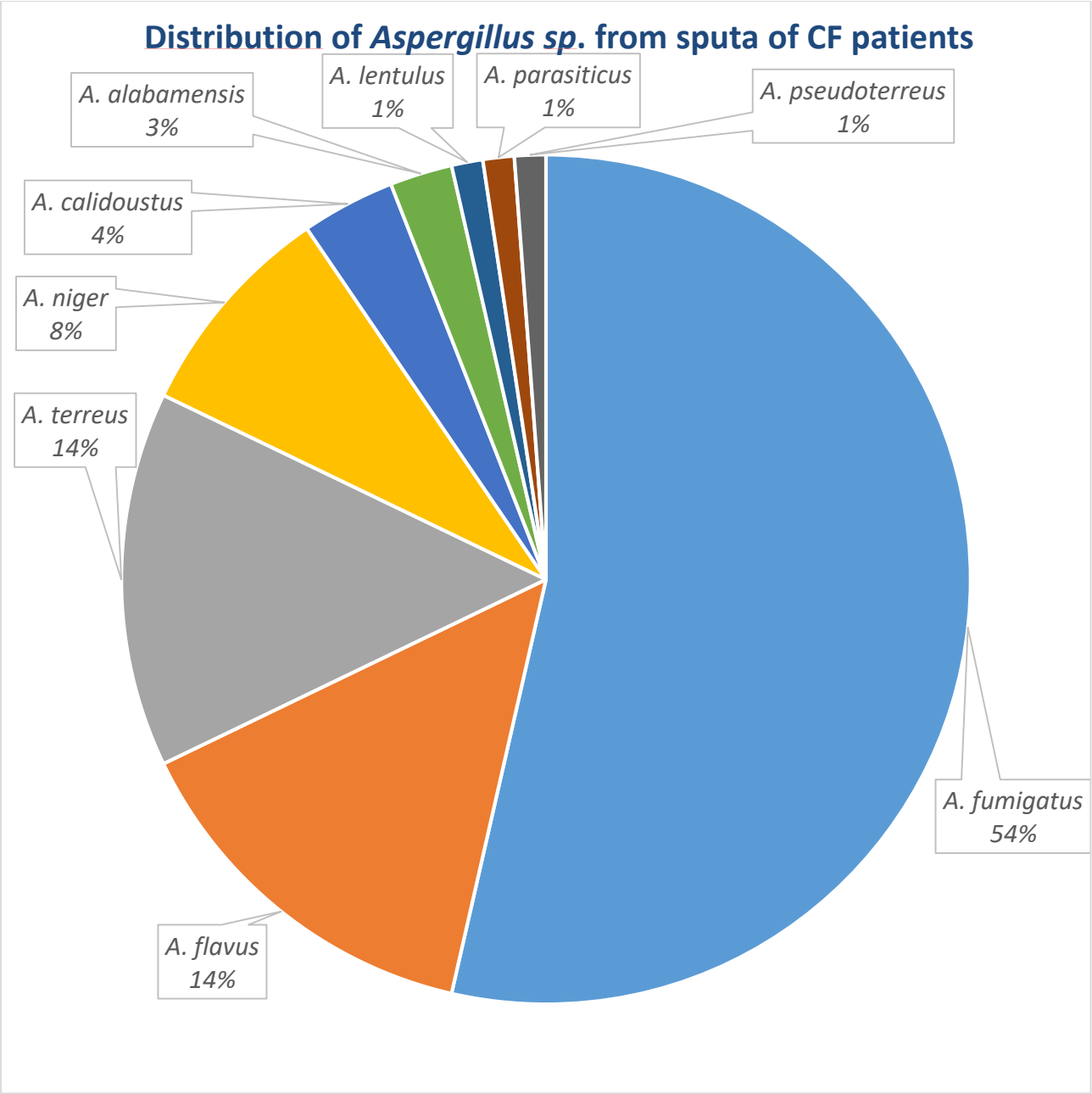

Figure 2 – Distribution of *Aspergillus sp*. isolated from sputa of CF patients. All isolates were identified by beta-tubulin and Calmodulin sequencing and phylogenetic analysis. Nevertheless, it was not possible to differentiate *A. Flavus* from *A. oryzae* with this method. In such cases, all isolates were annotated as *A. flavus*, until further characterization allows proper identification.

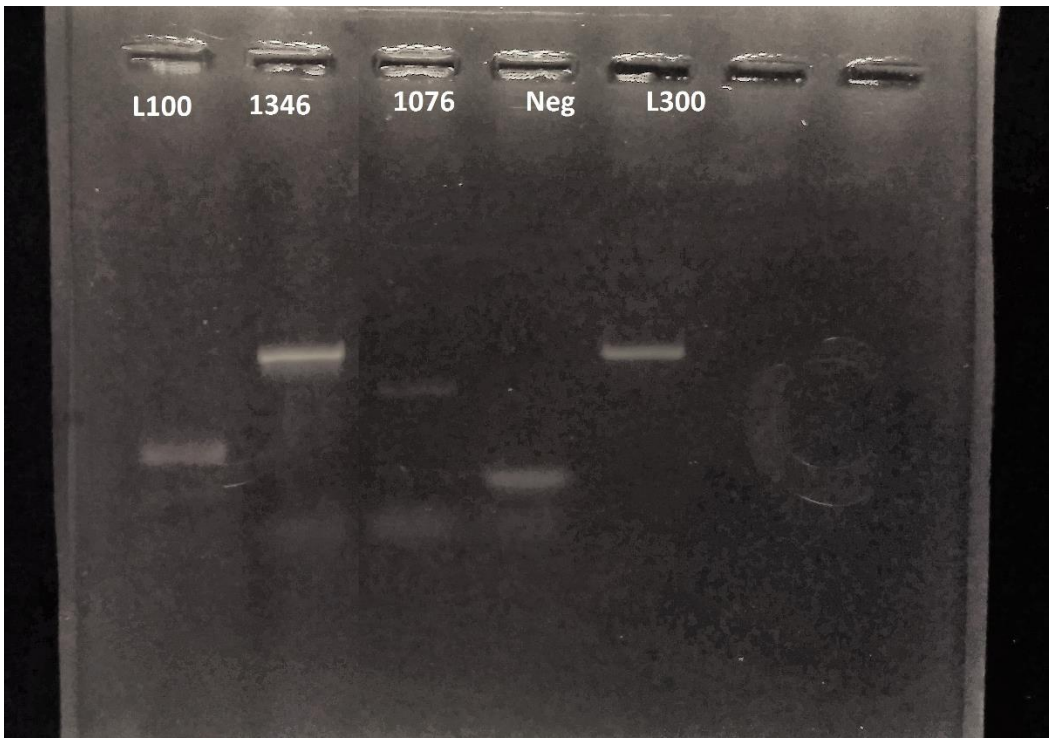

Figure 3 – Amplicons of the multiplex-PCR for identification of *Scedosporium species*. Lane1: 100pb DNA ladder; Lane 2: *S. apiospermum* as confirmed by beta-tubulin sequencing. Lane 3: S. prolificans; Lane 4: PCR negative control; Lane 5: 300pb DNA ladder.

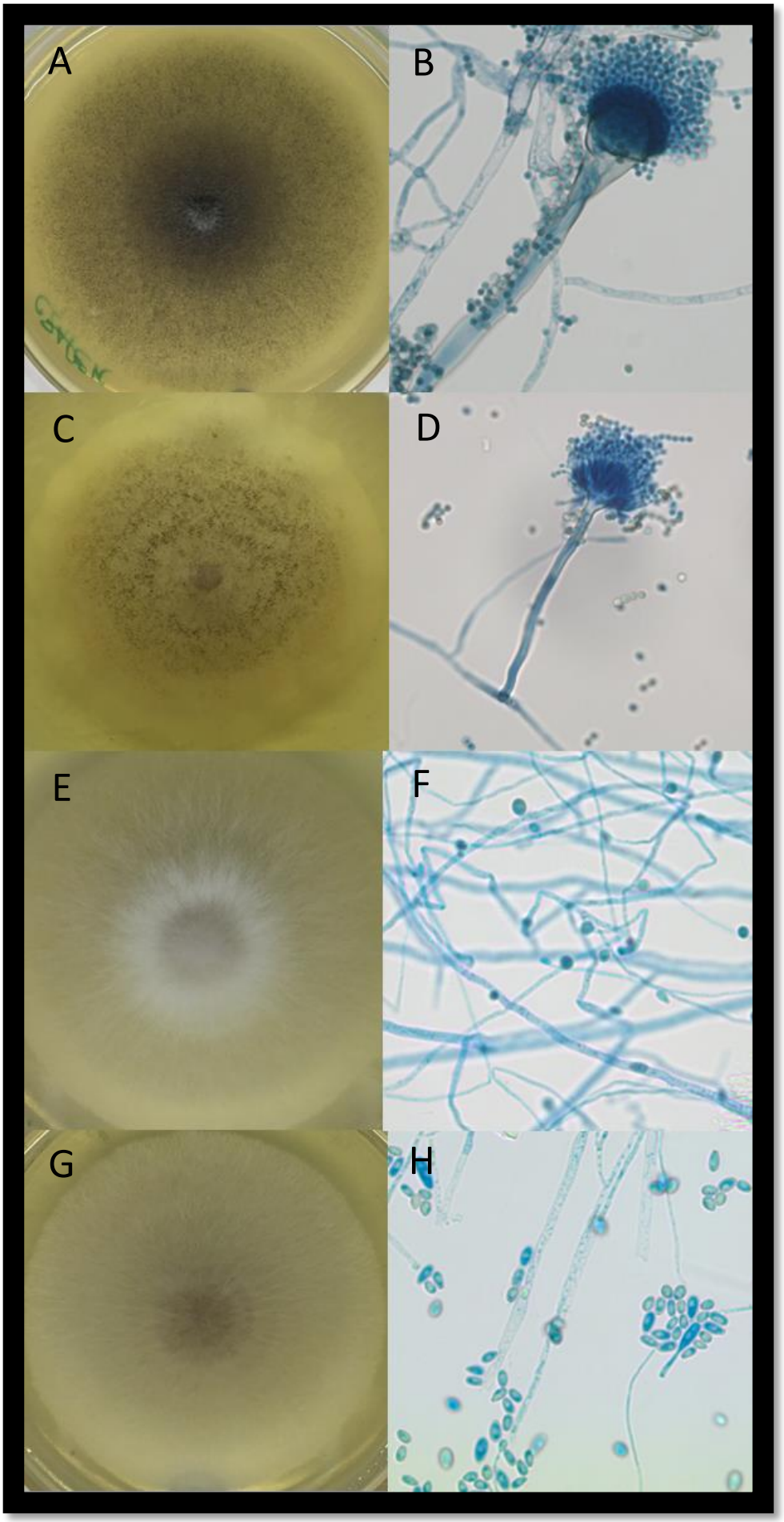
A
B
C
D
E
F
G
H

Figure 4 – Macro and micromorphology images of fungal isolates after growing them on Czpaek agar plates for 7 days and on PDA, respectively. A/B = *A. fumigatus*. C/D = *A. flavus*. E/F = *S. apiospermum*. G/H = *S. aurantiacum*.

Conclusion

Improvement to the life expectancy of CF patients brings about novel challenges including the need for evaluation of the role of fungi in the CF lung. The prevalence and importance of *fungi* in the CF airway has likely been underestimated.

This is the first report about fungal infections in patients with CF in Argentina. *A. fumigatus* was the most prevalent hyphomycete in CF samples. Although less common, several fungal species including *A. alabamensis* and *A. calidoustus* may be isolated transiently from CF respiratory secretions, while others such as *A. fumigatus*, *A. terreus*, *E. dermatitidis*, *S. apiospermum* and *S. aurantiacum* may chronically colonize the airways. Further studies are needed to evaluate the contribution of the different fungi to inflammation and clinical deterioration in CF patients and the clinical implications of fungal coinfections in the physiopathology of CF lung infection-colonization.

Finally, research in this emerging field is warranted to fully elucidate the indications for therapeutic interventions.

References

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