

Correlation between classical and molecular methods to identify moulds from clinical samples

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INTRODUCTION

Advances in molecular tools have yielded a multitude of genetic studies rendering many nomenclature and taxonomical changes within pathogenic fungi. Several species have been synonymized and others have been divided into several taxa. Thus, *Aspergillus fumigatus* or *Scedosporium apiospermum* are now a complex of several species that are almost indistinguishable by classical morphological methods. These species are considered as cryptic and can only be confidently identified by molecular methods.

Although developments in identification tools, such as MALDI, are becoming available for some centers many others still rely in morphological identification specially for moulds. The aim of this study was to analyze the correlation between morphological and molecular identification of moulds.

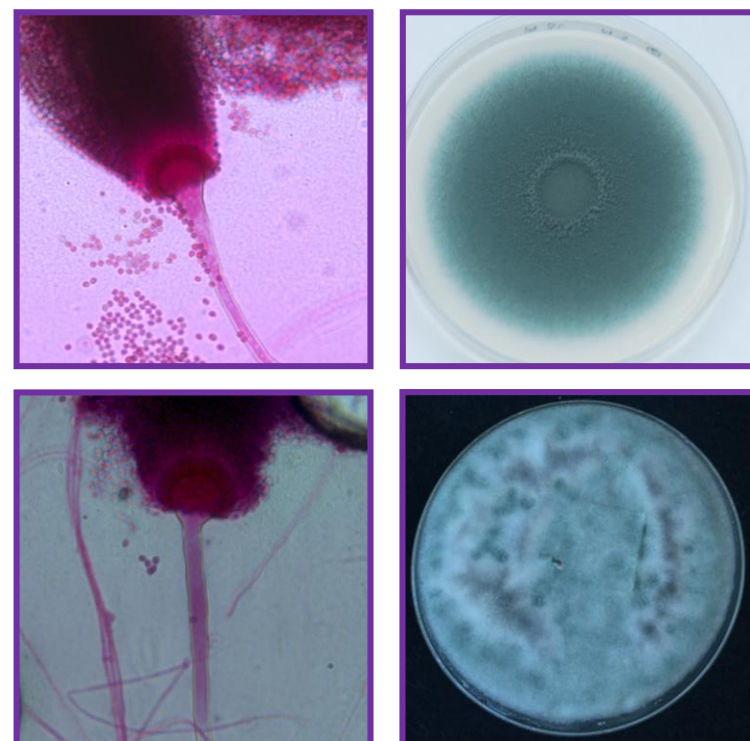


Figure 1. Micro and macro morphology of *A. fumigatus* (up) and *A. lentulus* (down)

METHODS

Three hundred and twenty two strains isolated from a population based survey study (FILPOP¹) performed in thirty Spanish hospitals were analyzed.

The identification by morphological methods was performed in each hospital. The molecular identification was performed in the reference laboratory. Internal Transcribed Spacer regions were sequenced for all strains. In addition to reach species level part of the beta tubulin gene was sequenced for *Aspergillus* spp. and *Scedosporium* spp. and part of the elongation factor alpha for *Fusarium* spp. The agreement between both methods was calculated. When the classification was not in agreement in both methods the following criteria was used to classify the errors in morphological identification: Strains only identified to genus level or belonging to the same species complex were considered Minor Errors (MiE), strains classified in a different species complex were classified as Major Errors (ME), strains identified in a different genus were classified as Very Major Errors (VME).

RESULTS

The agreement between morphological and molecular methods was 65.8%. Two hundred and twelve strains were correctly classified by morphological methods. Seventy four (23%) strains were MiE (46 were classified within the correct species complex and 28 only classified to genus level by morphology). Fourteen strains (4.3%) were ME (*Aspergillus* strains classified within a different species complex by molecular and morphological methods). Ten identifications (3.1%) were VME (classified in a different genus by morphological and molecular methods). Twelve strains (3.7%) were reported by the hospitals as “unidentifiable”, “non-sporulating”, “mucoral” or “mould” and were not classified in the error categories.

Table 1. Number of strains and percentage in each of the groups defined

Group No (%)	OK	MiE	ME	VME	NA	Total
<i>Aspergillus</i> spp.	201 (72.3%)	55 (19.8%)	14 (5%)	2 (0.7%)	6 (2.2%)	278
<i>Scedosporium</i> spp.	8 (53.3%)	5 (33.3%)	-	1 (6.7%)	1 (6.7%)	15
Mucorales	2 (16.7%)	7 (58.3%)	-	2 (16.7%)	1 (8.3%)	12
<i>Penicillium</i> spp.	-	4 (57.3%)	-	2 (28.6%)	1 (14.3%)	7
<i>Fusarium</i> spp.	1 (25%)	2 (50%)	-	-	1 (25%)	4
Others*	-	1 (16.7%)	-	3 (50%)	2 (33.3%)	6
Total	212 (65.8%)	74 (23%)	14 (4.3%)	10 (3.1%)	12 (3.7%)	322

* Including Species from genera: *Cladosporium*, *Psathyrella*, *Arthrinium*, *Phialophora*, *Scopulariopsis* and *Paecilomyces*

Conclusions

- Classical methods were unable to classify to species level 34.2% of the strains analyzed in this study.
- Most of the *Aspergillus* strains were correctly classified to species level by morphology, being most of the errors in cryptic species.
- The misidentification of a cryptic species can be critical for those with a more resistant phenotype such as *Aspergillus lentulus*.
- The identification of Non-*Aspergillus* species produce more identification errors, including high percentage of VME. This could translate in important implications in the clinical practice because of the different susceptibility profile of these species.
- In samples isolated from deep infections additional tools like, molecular identification or antifungal susceptibility profile should be used for a correct management of the patients.

Reference

1. Alastruey-Izquierdo A, Mellado E, Peláez T et al. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). AAC 2013 Jul;57(7):3380-7.

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