Prevalence of *Aspergillus* species in clinical samples isolates in the Reference Hospital Virgen del Rocío (Andalusia, Spain)

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**Background:**

Fungal infections are increasingly common; including those caused by species of the genus *Aspergillus*. The genus *Aspergillus* is divided in subgenera, and in sections. Within each section there are numerous cryptic species that can only be differentiated by molecular techniques. The prevalence and relevance of these cryptic species in the clinical setting are still unknown.

The aim of this study was to analyze the distribution of *Aspergillus* species among clinical samples isolates in the Hospital Virgen del Rocío (Spanish tertiary care hospital) collected between January-October 2014.

**Material/methods:**

**Strains:** all strains of *Aspergillus* species isolated from clinical samples, received in the hospital clinical microbiology laboratory over a period from January to October 2014, were included in the study. They were isolated from respiratory tract samples (n=119), otic exudates (n=29) and other locations (n=8).

**Morphological identification:** primary culture was performed using Sabouraud, Sabouraud-chloramphenicol and Sabouraud-cycloheximide agars (Oxoid); incubation at 25°C and 30°C, and twice a week examination for up to 3 weeks. Species identification of the isolates was done according to macroscopic and microscopic morphology.

**Molecular identification:** the strains were cultivated for 48 hours on Sabouraud chloramphenicol agar plates at 30°C and Genomic DNA was extracted with the QIAamp® DNA Mini Kit (Qiagen, Courtaboeuf, France). Identification of the fungal strains was performed by PCR amplification and DNA sequencing of the partial beta-tubulin (BT) gene using BT1 and BT4 primers and a BLAST search analysis (BLASTn) for species identification from the NCBI genomic database (http://blast.ncbi.nlm.nih.gov/) was conducted.

**Results:**

*Aspergillus* species were cultured from 156 samples belonging to 139 patients.

Attending to their macroscopic and microscopic characteristics, the isolates were identified as: 71 *A. fumigatus*, 39 *A. flavus*, 22 *A. niger*, 19 *A. terreus* and 5 *Aspergillus* spp.

DNA sequencing allowed us to detect cryptic species belonging to six different sections. *Aspergillus* section *Fumigati* included 71 (45.6%) strains of *A. fumigatus*. *Aspergillus* section *Flavi* was
represented by 40 (25.6%) strains: 36 A. flavus, 2 A. tamari, 1 A. minisclerotigenes and 1 A. nomius. *Aspergillus* section *Terrei* included 19 (12.2%) A. terreus strains. *Aspergillus* section *Nigri* included 22 (14.1%) strains: 12 A. niger and 10 A. tubingensis. The sections with fewer species were *Nidulantes* (3 A. nidulans strains) and *Versicolores* (1 A. sydowii strain).

The five *Aspergillus* spp. Strains were identified by sequencing as: 3 A. nidulans, 1 A. minisclerotigenes and 1 A. sydowii.

**Conclusions:**

*A. fumigatus* has been responsible for almost half of the infections as previously reported.

DNA sequencing allowed us to detect cryptic species belonging to six different sections and to classify 15 (9.6%) isolates of a total of 156 *Aspergillus* as cryptic species.