Title:

Aspergillus spp. prevalence in one Portuguese hospital. A reason to be worried?

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Aspergillus genus is responsible for over 80% of pulmonary invasive fungal infections in humans. Invasive aspergillosis, caused by *Aspergillus fumigatus* in 80% of the cases, is the most common invasive fungal infection.

The aim of this study was to assess prevalence of *Aspergillus* genus in a Portuguese hospital, using a wide sampling approach combining active and passive methods.

A total of 15 sampling sites were defined, distributed by the different hospital areas - emergency room, day hospital, internment ward, operating room and outpatient area. Active (air impaction) and passive (surfaces swabs, settled dust, filters from heating, ventilation and air conditioning (HVAC) systems and electrostatic dust collectors (EDC) sampling methods were applied. Samples were impacted/washed and seeded on: malt extract agar (MEA) supplemented with chloramphenicol (0.05%) and dichloran-glycerol agar (DG18).

The samples collected by air impaction presented the higher fungal diversity, yet *Aspergillus* sp. was not the most prevalent genera (2.69% MEA; 15.41% DG18). Within *Aspergillus* genus, section *Fumigati* was the one with highest prevalence in both culture media (86.67% MEA; 45.09% DG18) with other sections (*Aspergilli*, *Candidi*, *Nigri, Restricti, Versicolores* and *Usti*), presenting lower counts in both culture media.

Surface swabs were the only sampling method recovering *Aspergillus* section *Circumdati*, and on DG18. Concerning the settled dust samples, *Aspergillus* sp. was not the most prevalent (25% MEA; 9.26% DG18), with *Fumigati* section the only isolated on MEA and *Aspergilli* (35.71%) *and Versicolores* (64.82%) sections on DG18. On the EDC and HVAC filters samples *Aspergillus* sp. was not found.

Overall, the results of this study highlight *Aspergillus* sp. presence in the assessed premises. The *Aspergillus* section *Fumigati*, with increased clinical relevance, was often detected in this clinical environment. The observed levels and distribution of *Aspergillus* suggest the need to implement corrective measures not only due to *Aspergillus* counts, but also due to the toxigenic potential of some *Aspergillus* sections. The different fungal prevalence found with the different methods applied, validates the utility of having multiple sampling strategies when defining a regular routine assessment in clinical environments.

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