

Nasal swab as a tool to access occupational exposure to fungi in a cork industry

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Approximately 49% of all cork produced worldwide in 2016 was from Portugal, where there are 650 companies working in this production sector (APCOR, 2016). Additionally, two thirds of the world exportation comes from Portugal, with Portuguese cork industry employing about ten thousands workers (MTSS 2009). The presence of the *Penicillium* section *Aspergilloides* (formerly known as *Penicillium glabrum*) in this industry involves the risk of respiratory diseases such as suberosis, a type of hypersensitivity pneumonitis that is one of the most prevalent diseases among cork workers (Oliveira et al. 2003). Besides *Penicillium* section *Aspergilloides*, *Aspergillus* section *Fumigati* was also reported in cork industries (Viegas et al. 2015). This is one of the most ubiquitous saprophytic fungi and is also considered one of the potential pathogenic species with highest clinical relevance (Dagenais and Keller 2009). The use of the nasal swab procedure is of particular importance since it allows to determine fungal presence in the nose cavity, being an easy and painless collection method (Heikkinen et al. 2002). The aim of this study was to determine the exposure to the dominant mycobiota of this occupational environment through the mycological analysis of nasal exudates from cork industry workers.

Nasal mucous samples from 360 workers from 3 companies (plant A - 41workers; plant B – 165 workers; and plant C – 154 workers) and also from a control group (38 individuals) with administrative tasks were performed. Duplicate samples were taken with sterilized cotton swabs from one nostril of each worker. The swabs were rotated against the internal anterior walls of the nostril and then placed in the provided transport tube. One of the swab samples was then plated onto malt extract agar (MEA) supplemented with chloramphenicol (0.05 %). All the collected samples were incubated at 27 °C for 5 to 7 days. The other swab sample was used for DNA extraction following molecular identification of *Penicillium* section *Aspergilloides* and *Aspergillus* section *Fumigati* by Real Time PCR (qPCR).

Among the 360 workers subjected to the nasal swab assay only 50 (13.9%) did not present fungal contamination. Around 36.6% of the workers nasal swabs presented *Penicillium* genus contamination, 9.9% with *Aspergillus* sp. and 29.1% with more than one fungal genera. Among the 38 subjects from the control group, 16 (42.1%) did not present fungal contamination, 44.7% present *Penicillium* sp. and 18.4% *Cladosporium* sp. One subject presented *Mucor* sp. and other *Geotrichum* sp. contamination. DNA from *Penicillium* section *Aspergilloides* was successfully amplified by qPCR in 37 cork workers. From those, it was only possible to identify in 12 samples the genus *Penicillium* by culture based-methods. *Aspergillus* section *Fumigati* was also co-amplified with *Penicillium* section *Aspergilloides* in one worker, while in another one was detected singularly. As expected, in the 38 controls analysed none were positive for *Penicillium* section *Aspergilloides* nor *Aspergillus* section *Fumigati*.

The fungal species identified and detected in the collected nose swabs presented the same trend described for this very specific occupational environment. This approach allowed us to estimate the risk associated with the tasks performance, since high dust contamination is expected to promote the exposure to fungi playing a role as carriers to the worker's nose. As observed in previous environmental assessments, culture-based methods coupled with molecular tools allowed to obtain a wider spectrum of the workers nasal mycobiota.

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Main topic (tick the key topic of your presentation) :

- Effects of biological agents on the health of workers exposed: infectiology and toxicology research, epidemiological studies, dose-response relationships, etc.
- Methods and strategies for the qualitative and quantitative assessment of biological risks: risk assessment methods, biometrology, methods and strategies for exposure measurement (bioaerosols, liquids, surfaces, real time), research resources (atmosphere generation, modelling), data interpretation, etc.
- Exposure to biological agents at the workstation: sectors and biological agents concerned, emission sources and exposure situations, characteristics of exposure (concentration, particle size distribution and biodiversity of bioaerosols, etc.), multi-exposure, biometrology, etc.
- Prevention measures: means available for reducing exposure, ventilation, innovative processes, personal protection, new technologies for bioaerosol removal and surface cleaning, etc.

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