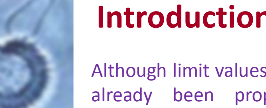
Molecular biology *versus* conventional methods

Complementary methodologies to understand occupational exposure to fungi

Carla Viegas ESTeSL
carla.viegas@estesl.ipl.pt
Joana Malta-Vacas ESTeSL
Raquel Sabino INSA



Introduction

Although limit values for fungal air contamination have already been proposed, these values are not consensual because of the lack of uniformity in the environmental monitoring procedures and laboratory methods.
(Green *et al*. 2006)

Exclusive use of conventional methods may underestimate the results due to different reasons:

- incubation temperature will not be the most suitable for every fungal (Zorman & Jerseck, 2008);
- fungal species with higher growth rates may inhibit others species' growth;
- non-viable fungal particles that may have been collected (Bartlett *et al*. 2004; Strachan *et al*. 1990).

Introduction

Despite molecular methods being more sensitive, specific and fast, conventional methods are still needed when it is necessary to characterize the fungal distribution in poorly studied settings.
(Stetzenbach *et al*. 2004)

The use of molecular methods requires a prior knowledge of the genome of the target species/ strains.
(Douwes *et al*. 2003)

Available genetic information is still scarce and scattered for many fungal species and costs associated with molecular biology techniques are still high.
(Horner, 2003)

In parallel with molecular biology, the use of conventional methods is still recommended for fungal species confirmation.
(Borman, 2009)

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Introduction

Confirmed presence of the *Aspergillus flavus* and *Aspergillus fumigatus* species requires implementation of corrective measures.
(AIHA, in 1996)

- *Aspergillus fumigatus* is responsible for severe aspergillosis, sometimes leading to affected individuals death.
(Yao & Mainelis, 2007)

- *Aspergillus flavus* is a complex of species known to produce mycotoxins (e.g. aflatoxin) some with proven carcinogenic potential.
(AIHA, 1996)

Introduction

Study developed in 7 poultries and 7 swineries.

This study aimed to determine:

- fungal distribution
- understand the occupational exposure to fungi belonging to *Aspergillus fumigatus* and *Aspergillus flavus* complex in a poultry farm.

Conventional and molecular methods were used in order to obtain results that will allow a more complete intervention and a more effective improvements of the working conditions in this setting.

Materials and methods

A descriptive study was conducted in a poultry farm in order to ascertain the fungal air contamination using conventional and molecular methods.

Measurements were carried out in January 2011 in two pavilions (P1 and P2) of the referred poultry.

Fig. 1- Pavillion indoor

Materials and methods

CONVENTIONAL METHODOLOGIES

Six air samples of 25 L were collected with the Millipore Air Tester (Millipore) by impact method at a velocity of 140 L / minute and at one meter height, using malt extract agar supplemented with chloramphenicol (0.5%) to inhibit bacterial growth.

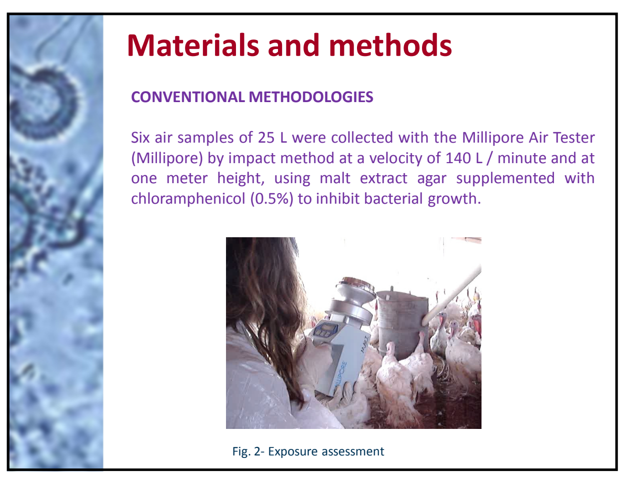


Fig. 2- Exposure assessment

Materials and methods

CONVENTIONAL METHODOLOGIES

Identification of filamentous fungi was carried out by macroscopic examination of the culture and microscopic observation of the fungal material mounted in lactophenol blue stain and achieved through morphological characteristics listed in illustrated literature.

(Hoog *et al*. 2000)

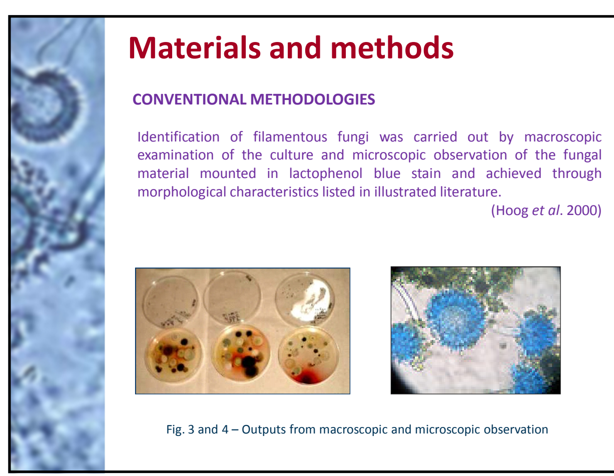


Fig. 3 and 4 – Outputs from macroscopic and microscopic observation

Materials and methods

MOLECULAR ANALYSIS

Air samples were collected using the Coriolis µ air sampler. Each sample consisted of 300 L of air, collected at 300 L/min.

DNA was then extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research) according to the manufacturer's recommendations.

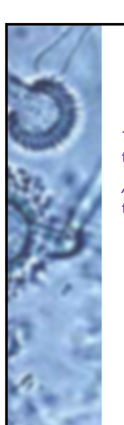


Fig. 5 – Exposure assessment - Coriolis

Samples used for the two laboratory approaches, were collected simultaneously in the two pavilions of the studied poultry farm, indoors and outdoors. Outdoor samples were considered as the reference samples.

Results and discussion

CONVENTIONAL METHODS

72.0% of the isolates belong to *A. flavus* complex.

A. flavus were isolated in all the air samples collected.

A. fumigatus complex were not detected in the six air samples collected, but its presence was confirmed inside the two pavilions in other types of samples (litter and surfaces).

MOLECULAR METHODS

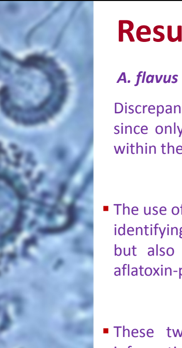
Only two of the six analyzed air samples were positive to species belonging to *Aspergillus flavus* complex, corresponding to the indoors and outdoor air samples from Pavilion 2.

For *Aspergillus fumigatus* complex, showed positive results in three of the samples.

Fig. 6 – PCR equipment

Results and discussion

Table 1 – Outputs from conventional and molecular methods



Sample description	A. sFumigati		A.sFlavi	
	CFU/m³	RT-PCR	CFU/m³	RT-PCR
Inside P1, 1st floor entrance	0	-	>2000	-
Inside P1, 1st floor	0	-	>2000	-
Inside P1, ground floor	0	+	1600	-
Outside P1	0	-	1040	-
Other samples	+	N/A	+	N/A
Inside P2	0	+	200	+
Outside P2	0	+	20	+
Other samples	+	N/A	+	N/A

Results and discussion

A. flavus

Discrepancy it can be explained by the molecular strategy adopted, since only toxigenic strains (aflatoxin-producing) were investigated within the group of species belonging to *Aspergillus flavus* complex.

- The use of both methodologies allowed us to establish a strategy for identifying a potential exposure of the not only to fungal particles, but also to the possible presence of mycotoxins produced by aflatoxin-producing species.
- These two methodologies used in parallel provide additional information useful in the evaluation of occupational exposure.

Results and discussion

The lack of positive cultures belonging to **Fumigati section**, may be due to growth inhibition caused by other species present in culture with higher growth rates.

(Bartlett *et al*. 2004; Strachan *et al*. 1990)

When using molecular methodologies, any fungal particles can be detected, regardless their state of growth or even viability. This allows the sampling of a larger air volume, obtaining a more representative sample regarding fungal composition of the environment.

In the case of a possible fungal occupational exposure through inhalation, conventional methods offer the advantage of enabling identification and quantification only of viable microorganisms and, therefore, the ones causing higher risk for workers health.

(Samson *et al*. 2000)

Results and discussion

In one of the pavilions, three indoor samples had a higher fungal burden than outdoor and the other pavilion's outdoor sample presented higher levels of CFU/m^3.

Regarding qualitative comparison of both environments, only one species was different from the ones isolated from outdoors (*Rhizopus* sp.).

When different species are detected between indoor and outdoor air, molecular biology can complement the conventional methods used to identify potentially toxinogenic strains and to confirm results of a possible fungal contamination from internal sources.

In the poultry farms case, these sources may be various, including birds, floor cover, food or other specific activities as changing the floor covering. (scheff *et al*. 2000)

Conclusions

Application of conventional methods allows the characterization of the composition of fungal flora in a poultry farm.

By comparing data, quantitatively and qualitatively, with the reference site (outdoor air sample), this method allows the assessment of air fungal contamination in the studied setting.

The use of complementary molecular methods allows the suppression of some limitations of the cultural methods, detecting the presence of non-viable particles and identifying potential mycotoxin producers' strains.