

Slaughterhouses fungal burden assessment: A contribution for the pursuit of a better assessment strategy

Bioaerosols are comprised of airborne bacteria, fungi, viruses and their by-products, endotoxins and mycotoxins. Fungal spores are complex agents that may contain multiple hazardous components. Health hazards may differ across species and strains because fungi may produce different allergens and also mycotoxins, and some species can infect humans. In slaughterhouses the biological risk is present not only from the direct or indirect contact with animal matter but also from the exposure to bioaerosols.

This study intended to assess fungal contamination by cultural and molecular methods in poultry; swine/bovine; and large animal (bovine and horses) slaughterhouses.

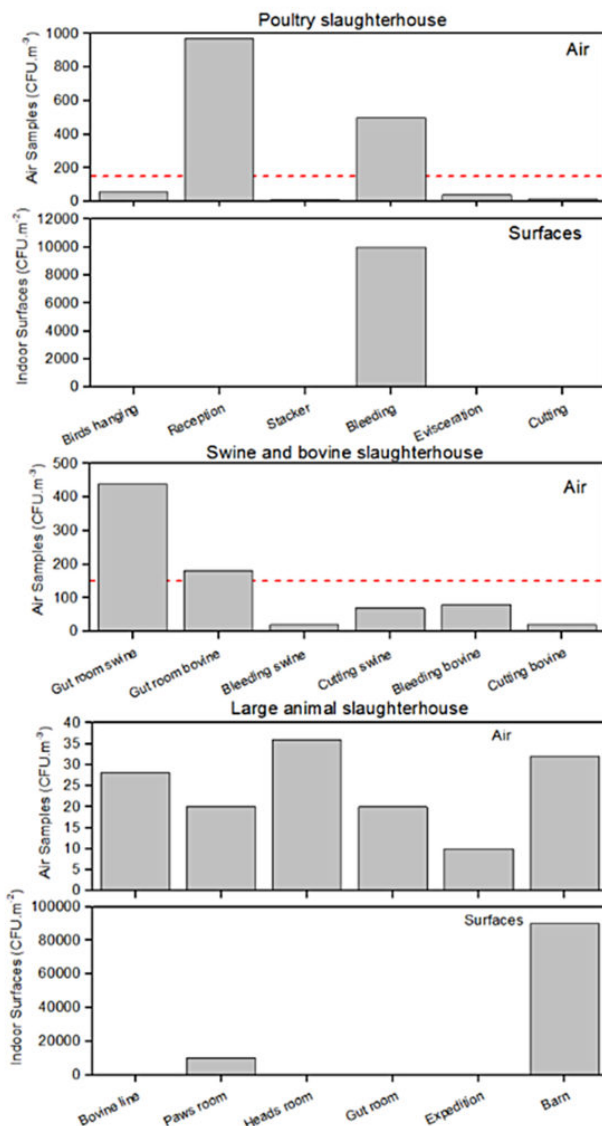


Fig. 1. Fungal load distribution in the three assessed slaughterhouses. Dashed line represents reference limits suggested by World Health Organization (WHO).

Three slaughterhouses were assessed between January and June from 2015 during a normal working day. One poultry slaughterhouse, one swine and bovine slaughterhouse and one large animal slaughterhouse were selected. Air samples of 100L to 250L were collected through an impaction method, a process whereby particles are removed from an air stream. Surfaces samples were collected in parallel. Additionally, extra air samples of 300L were collected to perform molecular identification of different fungal species/strains selected due to their clinical relevance and toxigenic potential, namely *Aspergillus* sections *Fumigati*, *Flavi* and *Circumdati*.

Air fungal load ranged from 16 to 970 CFU/m³ in poultry slaughterhouse to 20 to 440 CFU/m³ in swine/ bovine slaughterhouse and 10 to 36 CFU/m³ in large animal slaughterhouse (Figure 1). The surfaces presented results that ranged from 0 to 10000 CFU/m² in the poultry slaughterhouse and 0 to 90000 CFU/m² in the large animal slaughterhouse. No fungal isolates were found in swine/bovine slaughterhouse surfaces (Fig. 1). Poultry and swine and bovine slaughterhouses presented each 2 sampling sites that surpassed the guideline proposed by World Health Organization (WHO) (maximum value of 150 CFU/m³). Table 1 shows that *Scopulariopsis candida* was the most frequently isolated species (59.5%) in the poultry slaughterhouse air, while *Cladosporium* sp. was most abundant (45.7%) in swine/bovine slaughterhouse; and *Penicillium* sp. prevailed (80.8%) in the large animal slaughterhouse. Six fungal species were found only in surfaces samples. Molecular tools amplified successfully DNA from the *Aspergillus* section *Fumigati* in six sampling sites where the presence of this fungal species was not identified by conventional methods.

Poultry slaughterhouse presented the most critical fungal load, not only because two sampling sites that surpassed the selected guideline, but also due to the fact that all air samples presented a higher indoor fungal load when compared with the outdoor samples, strongly suggesting the presence of indoor fungal contamination sources. However, we must point out that viable bioaerosol particles constitute a small percentage of the total concentration of microorganisms, and therefore, we should consider a bias regarding fungal burden in all units assessed. One of the most important stage of risk control is to verify the existence of indicator species/strains that are representative of harmful fungal contamination in the analyzed setting. For that, it is important to identify what are the best indicators for slaughterhouses occupational environment. The detection of one *Aspergillus* species complex in sampling sites where it wasn't isolated by conventional methods corroborate the importance to combine both methodologies in parallel.

| Indoor air samples (PS) | (CFU/m³; %) |
|--------------------------------------|-------------------------------|
| <i>Scopulariopsis</i> sp. | 950; 59.52 |
| <i>Penicillium</i> sp. | 524; 32.83 |
| <i>Aspergillus fumigatus</i> complex | 50; 3.13 |
| Others | 72; 4.5 |
| Indoor surfaces samples (PS) | (CFU/m²; %) |
| <i>Mucor</i> sp. | 10000; 100 |
| Indoor air samples (SBS) | (CFU/m³; %) |
| <i>Cladosporium</i> sp. | 370; 45.7 |
| <i>Penicillium</i> sp. | 270; 33.3 |
| <i>Aureobasidium</i> sp. | 90; 11.1 |
| Others | 80; 9.9 |
| Indoor air samples (LAS) | (CFU/m³; %) |
| <i>Penicillium</i> sp. | 118; 80.8 |
| <i>Aspergillus ochraceus</i> complex | 8; 5.5 |
| <i>Cladosporium</i> sp. | 8; 5.5 |
| Others | 12; 8.2 |
| Indoor surfaces samples (LAS) | (CFU/m²; %) |
| <i>Scopulariopsis brumpti</i> | 40000; 40 |
| <i>A. terreus</i> complex | 30000; 30 |
| Others | 30000; 30 |

Tab. 1. Most common fungi isolated in the three slaughterhouses

In addition to providing information regarding fungal contamination background in slaughterhouses occupational environment, this study identifies the fungal species most representative of harmful contamination. The strategy of using cultural-based methods and molecular tools in parallel as a protocol to ensure a proper characterization of fungal contamination occupational exposure is also an added value of this assessment. Moreover, this specific occupational environment is a good example of the reality of occupational exposures: co-exposure to several risk factors by different exposure routes. This justifies special caution when comparing exposure results with occupational exposure limits and also when considering the cumulative risk assessment as the best option when performing risk assessment.

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