Caracterização do microbiota em habitações Portuguesas
Bioburden characterization in Portuguese dwellings

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Resumo

O microbiota presente em ambientes interiores está associado a riscos para a saúde dos ocupantes, como asma, alergias e desconforto respiratório, aumentando a importância da sua caracterização nestes ambientes. Realizou-se um estudo com o objetivo de caracterizar o microbiota de 23 casas situadas na região de Aveiro. Como método de amostragem passivo, foram usados precipitadores electrostáticos. A concentração fúngica variou entre 448 e 74742 CFU/m^{2}e a bacteriana variou entre 249 e 31548 CFU/m^2. O Quarto foi o local amostrado que apresentou maior contaminação. O método de amostragem passiva utilizada deverá ser considerado em estudos futuros para avaliar o risco em habitações de doentes imunocomprometidos ou outras populações susceptíveis.

Palavras-chave: *Microbiota; Casas; EDC; Fungos; Bactérias*

Abstract

The microbiota present in indoor environments is associated with occupant health risks, such as asthma, allergies and respiratory discomfort, increasing the characterization importance in these environments. A study was performed to characterize the microbiota of 23 dwellings located in the region of Aveiro. As a passive sampling method, electrostatic dust cloths (EDC) were used. Fungal levels ranged from 448 to 74742 CFU/m^2 and bacteria from 249 to 31548 CFU/m^2. The room was the sampling site with higher contamination. The applied sampling method should be taken into consideration in future risk assessment studies at homes of immunocompromised patients and other susceptible populations.

Keywords: *Bioburden; Dwellings; EDC; Fungi; Bacteria.*

1. Theory

According to WHO, 4.3 million people die each year from exposure to domestic air pollution. Currently, people spend more than 90% of the day

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indoors in their own houses or in work place (Lee et al., 2001), so it is increasingly important to study indoor air quality.

Organic dust consists of particulate matter with microbial, vegetable or animal origin. Its specific agents include viruses, bacteria, gram negative endotoxins, actinomycetes, fungi, mycotoxins, algae or plant cells, enzymes and proteins from plants or animals, antibiotics or other products from other processes, insects and mites (and their fragments and fragments) (Lacey et al., 1994; Douwes et al., 2003; Eduard et al., 2012; Sturm, 2016; Viegas et al., 2018). The bioburden present in organic dust, comprising fungi and bacteria, should be measured and distribution characterized.

Conducting indoor air quality (IAQ) studies in several buildings has shown that occupants with contaminated indoor air generally exhibit symptoms of lethargy or fatigue, headaches, dizziness, vomiting, difficulty concentrating and other symptoms (Verhoeff et al., 1997; European Environment and Health Information System, 2007; Shenassa et al., 2007). The collection of particulate matter inside buildings is commonly used for studies linking human health to disease (Adams et al., 2015). It is known that the level of indoor air pollution can reach values two to five times higher than outside air, and poor air quality affects general well-being, comfort and has health effects, in particularly in the respiratory system. It is also linked to cardiovascular and oncological diseases due to specific pollutants (Adams et al.,2015).

Based on previous studies *Cladosporium, Penicillium and Aspergillus* genera are the most prevalent identified indoors (Ayanbimpe et al.,2010; Mentese et al., 2009). *Micrococcus* spp., *Staphylococcus auricularis*, and the gram-negative bacteria *Bacillus* spp. were the most prevalent found in indoors concerning the bacteriota (Mentese et al., 2009).

A passive method of collecting dust began to be more commonly applied, mainly for IAQ evaluations, known as electrostatic dust cloth (EDC). EDC is an easy-to-use, inexpensive passive sampling method consisting of an electrostatic polypropylene cloth (American Conference of Governmental Industrial Hygienists, 2009; Kilburg-Basnyat et al., 2016; Viegas et al., 2018). EDCs have electrical fibers that increase the retention of particles with allergic characteristics (Kilburg-Basnyat et al., 2016; Viegas, et al., 2017; Viegas, et al., 2018) and if placed on the correct and raised surface, it allows the correct and efficient collection of the dust present in the air (Normand et al., 2009; Kilburg-Basnyat et al., 2016).

The main objective of this study was to assess the bioburden of 23 dwellings in Aveiro through EDCs.

2. <u>Methodology</u>

2.1. Sampling Locations

This study was conducted between September 2017 and June of 2018 in twenty three Portuguese dwellings. While being part of a larger study in which additional environmental characterization was carried out, this paper presents the preliminary results regarding samples collected by EDC. All the dwellings analyzed, were located in the center of Aveiro (northwest of Portugal) (figure 1). The sampling sites were the same for the 23 houses, since one EDC was placed in the following 3 divisions: kitchen, living room and bedroom.

Figure 1: Location of dwellings in Aveiro

2.2 Electrostatic Dust Cloth (EDC) Extraction and Bioburden Characterization

Each EDC have a surface exposure area of 0.0209 m and dust was allowed to settle on average for 30 days. Each EDC was weighed after sampling, and then the weight of the blank (0,0 g) was subtracted to determine the mass of the collected dust. Each EDC cloth was washed with 20 mL 0.9% NaCl with 0.05% Tween80™ (Merck S.A, Lisbon, Portugal) by orbital shaking (250 rpm, 60 min, at room temperature), and 150 _L of the wash suspension was inoculated on to 4 different culture media: media: 2% malt extract agar (MEA) with 0.05 g/L chloramphenicol media; dichloran glycerol (DG18) agar-based media; tryptic soy agar (TSA) with 0.2% nystatin; violet red bile agar (VRBA). The following procedures were described elsewhere (Viegas et al., 2018)

3. <u>Evidence</u>

3.1 Bioburden - Bioburden assessment

On the MEA media fungal contamination ranged from 448 to 74742 CFU/m^2. The dwelling with the highest burden was the number 15, followed by the number 3 (Figure 1).

Concerning the DG18 media fungal contamination ranged from 0 to 74743 CFU/m^2 and the house that presented the highest burden was the house 15, followed by 16 and 17 houses both presenting 74643 CFU/m^2.

Figure 2 – Fungal distribution in the 23 dwellings assessed by EDC

Bacterial concentration ranged from 249 to 31548 CFU/m^2 and the maximum value belong to the dwelling 13. Gram-negative bacteria concentration ranged from 0 to 746 CFU/m^2 with the highest load corresponding to the 13.

Figure 3 - Bacterial distribution in the 23 dwellings assessed by EDC

3.2 Bioburden distribution

The highest median fungal concentration was observed in the Room (24880 CFU/m^2), followed by Kitchen and Living room with 1393 and 348 CFU/m^2 respectively.

The site with the highest median of total bacterial load was the Room and Living room with the same value (348 CFU/m^2) and for gram-negative bacteria was 697 CFU/m^2 in Room followed by the kitchen with 199 CFU/m^2.

Table 1. Bioburden distribution on the assessed sampling sites

Sampling site	Statistics	Fungi (MEA) (CFU/m^2)	Fungi (DG18) (CFU/m^2)	Total bacteria (CFU/m^2)	Gram-negative bacteria (CFU/m^2)
Room	Median	24880	259	348	0
	Minimum	50	0	0	0
	Maximum	49761	26025	28911	697
Living Room	Median	348	846	348	0
	Minimum	0	0	0	0
	Maximum	26025	28911	2986	50
Kitchen	Median	1393	846	100	0
	Minimum	50	0	0	0
	Maximum	24881	28911	9256	199

3.3. Discussion

Unlike active sampling methods, such as air sampling, EDC can be applied inexpensively, without equipment and field staff, and collect integrated samples of bioburden over long-time periods (Kilburg-Basnyat et al., 2015). EDC passively collect bioburden while resting on flat surfaces in homes, schools, office buildings, agricultural, bakeries and industrial facilities (Noss et al., 2008; Samadi et al., 2010; Liebers et al., 2012; Jacobs et al., 2013; Viegas et al., 2018). The additional advantages of this method are the possibility of preparing sample dilutions during the laboratory procedure, overcoming the limitation of overloaded plates and easier the selection of selective culture media. In addition, being a passive collection method, the use of EDC allows sample collection over a longer period (ranging from weeks to several months), while air samples can only reflect the load over a period of time (mainly minutes) (Badyda et al., 2016; Viegas et al., 2017; Viegas et al., 2018).

Since bacteria and fungi need specific environmental conditions to grow and propagate, their levels are strongly affected by many factors, such as temperature and relative humidity (Mentese et al., 2009) justifying different contamination results in the assessed sampling sites. Fungal species occur in the presence of special substrates and are often found indoors rather than outdoors (Godish, 2001). The excessive humidity and/or high water content in building materials may be a promoting factor for microbial growth (WHO, 2009).

Different results were obtained from the two culture media applied for fungal assessment (MEA and DG18) allowing concluding about the importance of using both media to achieve a better characterization from the mycobiota (Viegas et al., 2018)

The sampling site with higher contamination was the Room, for both bacteria and fungi. These results are in accordance with other studies (Mentese et al., 2009) that also pointed the room as a sampling site with higher bioburden. This may be to the accumulation of clothes and the presence of carpets that increase the microorganism’s proliferation.

3.4. Conclusion

In light of the first results we can conclude that EDC was efficient as a suitable method for bioburden load characterization, since allowedto collect information from a larger period of sampling. Future studies should correlate dwellings characteristics with bioburden, in order to improve indoor air quality. Further studies should be also performed using this sampling method to monitor bioburden in dwellings from chronically immunocompromised patients, such as cystic fibrosis or cancer and transplant patients.

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