Indoor Allergens and Bacterial Assessment in Children Daycare Environments

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1. INTRODUCTION

Exposure and sensitization to indoor allergens are important risk factors for asthma and allergic respiratory diseases, playing a key role in triggering and exacerbating allergy and asthma symptoms in children (Salo et al., 2009). While children's greatest exposure to indoor allergens is at home, other public places where they spend a large amount of time, such as school and day care centers (DCCs), may also be sources of significant allergen encounters (Abramson et al., 2006). Moreover, the role of human occupancy as a source of indoor biological aerosol is poorly understood. Size-dependent particle behavior often can be associated with specific chemical and biological components of particulate matter. The strong signal of human microbiota, as airborne particulate matter is concerned, in an occupied room demonstrates that the aerosol route can be a source of exposure to microorganisms emitted from the skin, hair, nostrils and mouths of their occupants (Qian et al., 2012). The goal of this research was to determine human associated emission rates of bacteria and their possible relation with house dust mite and particle matter concentration in occupied DCCs classrooms.

2. MATERIALS AND METHODS

Nine children DCCs were invited to participate in this monitoring for house dust mites, total bacteria and particulate matter up to 10 micrometers in size (PM10). All the DCCs rooms were included in the assessment within 7 nurseries (< 1 year old children) and 45 kindergarten (1 to 5 year old children) classrooms.

2.1. Children day care centers

The assessment was carried out in 52 DCCs classrooms located in Porto urban area, both in summer (May – August 2011) and winter (November 2011 – February 2012) seasons, along one year study. Outdoor measurements were also conducted for total bacteria and PM10 monitoring in each campaign to compare with indoor levels.

2.2. Dust-mite assessment

Dust was collected in every room during classes and normal activities, using the same collection protocol in all sites. Samples were collected using a common vacuum cleaner equipped with a standardized collection sock filter fitted in the vacuum hose collector. About 3m² area, in the middle of the classroom, was vacuumed for a average period of 2 minutes. The selected areas had student activity or traffic. Samples were assayed for dust mite allergens, *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) with ELISA kits.

2.3 Particulate matter (PM10)

PM_{10} samples were collected using polytetrafluoroethylene (PTFE) filters on Personal Environmental Monitors (PEM) and Gilian personal pumps working at a flow rate of 2.0 $L.min^{-1}$, following US Environmental Protection Agency (EPA) Method 10-A, 'Determination of Respirable Particulate Matter in Indoor Air Using Size Specific Impaction' (Winberry et al., 1992). Pumps were calibrated and checked prior and after each sample, respectively, using a Gillian Gilibrator-2 Air Flow Calibrator. After weighting and before sampling, filters were stored in a desiccator. At least one field blank per sampling event was used. Exposed and unexposed filters were transported, protected from dust and sunlight, and kept away from air in a closed filter holder. Each filter was weighed under controlled temperature (19 to 22°C) and relative humidity (45 to 62%) before and after sampling using an electronic microbalance (Sartorius M5P with 0.001 mg of precision). Static charges were eliminated from filters using a non-radioactive, ionizing air blower (EXAIR, Model No. 7907). Concentrations were calculated by the filter weight and the respective sample air volume.

2.4 Total bacteria assessment

Air sampling was carried out with a microbiological air sampler (Merck Air Sampler MAS-100) using the culture media Tryptic Soy Agar (TSA) for total bacteria. It followed the National Institute for Occupational Safety and Health (NIOSH) 0800 Method - Bioaerosol Sampling (Indoor Air). Air was drawn at a rate of 100 $L.min^{-1}$at 1-1.5 m height; two different volumes of air (100 and 250 L) were drawn, according to the characteristics and hypothetic contamination of each room. Samples were collected both indoor and outdoor, in sequential duplicates, as well as one field blank per day. All samples were carried out during DCCs normal activities. Quantification of the collected samples was performed by naked eye count, after 48h incubation at 37°C. The concentrations obtained were expressed as number of colony forming units per cubic meter of air (CFU/m^3).

2.5 Statistical analysis

Descriptive statistics were calculated for allergens, bacteria and PM10 by season and DCC. Uncertainty was reported as 95% confidence intervals based on error propagation of multiple samples and instrumental uncertainty. Paired t-tests were used to test for seasonal effects differences and Pearson correlation test to identify possible associations between the analyzed parameters. A 0.05 level of significance was used for all analyses. All data were analyzed using IBM SPSS 20.0.

3. RESULTS AND DISCUSSION

3.1. Day care centers characterization

The 52 classrooms analyzed were all natural ventilated with two schools exceptions with AVAC systems, corresponding to 12 classrooms (23%). The number of occupants by classroom was in average 17 with a range of 5-29. The mean classroom area was 35 m^2 with a range of 12-59 m^2. Concerning physical parameters, the summer results for indoor temperature varied between 16°C to 25°C (average 19°C) and the RH varied from 30% to 79% (average 57%), with a mean air velocity of 0.04 $m.s^{-1}$. The winter indoor physical parameters ranged as followed: 13°C to 25°C (average 19°C) for temperature, 28% to 83% (average 56%) for RH and mean air velocity of 0.12 $m.s^{-1}$.

3.2. Environmental assessment

In our study the mean indoor total bacteria concentration is above the Portuguese reference levels (Table 1), being 58 and 53 times higher than outdoors, both in winter and summer respectively, which is in accordance with Hospodsky et al. (2012), revealing that human occupancy is a dominant factor that contributes to the concentration of indoor airborne bacterial genomes. Both resuspension from carpet and direct human shedding contributed to significantly elevate bacterial concentrations above background concentrations. Although the studied environmental parameters concentrations show some similar raising trends in winter season, the correlation coefficients between them were not significant ($r_{PM10/Der f1}$ = 0.042, P=0.782; $r_{Bacteria/Der f1}$ = 0.112, P=0.458).

Table 1. Descriptive statistics of indoor environmental assessment

	Minimum	Maximum	Mean	Std. Deviation	Reference
Bacteria Summer ($CFU.m^{-3}$)	190	44 230	5 476	6 606	500[a)]
Bacteria Winter ($CFU.m^{-3}$)	190	52 560	5 785	9 296	
Der f 1 Summer ($\mu g/g_{dust}$)	0.4	4.1	0.8	0.9	2[b)]
Der f 1 Winter ($\mu g/g_{dust}$)	-	249	5	35	
Der p 1 Summer ($\mu g/g_{dust}$)	0.4	1.5	0.6	0.3	
Der p 1 Winter ($\mu g/g_{dust}$)	0.4	11	1	2	
PM_{10} Summer ($mg.m^{-3}$)	0.04	0.4	0.1	0.08	0.15[a)]
PM_{10} Winter ($mg.m^{-3}$)	0.01	0.1	0.08	0.03	

a) Decree-Law No. 79/2006 of April 4th, Annex VII; b) Platts-Mills and De Weck, 1989.

4. CONCLUSIONS

Our results provide insights of inadequate indoor bacteria levels most likely due to human source contaminants that accumulate in the rooms. Nevertheless allergens and particles were not related with this indoor biological pollutant. Improvement in hygiene and ventilation measures may be advised to decrease the total bacteria levels in order to achieve a healthier indoor environment for children attending DCCs.

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