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# Airborne fungi in low and high allergic prevalence child care centers

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ABSTRACT

Fungi exposure has been linked to asthma and allergies among children. To determine the association between fungal exposure and wheeze and rhinitis symptoms, we examined concentrations of culturable indoor and outdoor fungi of various aerodynamic sizes in low and high allergic prevalence child care centers (CCCs) in Singapore. Environmental parameters were also performed for air temperature, relative humidity and ventilation rates, while information on CCC characteristics was collected via an inspection. Most commonly recovered fungi were *Penicillium*, *Aspergillus*, *Geotrichum*, *Cladosporium* and sterile mycelia with *Geotrichum* and sterile mycelia amounting to an average of 71.5% of the total airborne culturable fungi studied. Indoor and outdoor total culturable fungi concentrations and those in the size range of 1.1–3.3 μm were significantly higher in high allergic prevalence CCCs. When fungal types/genera were compared, indoor and outdoor *Geotrichum* and sterile mycelia of aerodynamic sizes 1.1–3.3 μm were found to be significantly elevated in high allergic prevalence CCCs. Indeed, average geometric mean diameters ($D_{g,\,ave}$) of indoor and outdoor culturable fungi were consistently smaller in CCCs with high prevalence of allergies than those with low prevalence. We found significant associations of higher fungal concentrations, especially those with smaller aerodynamic sizes in CCCs situated near parks. There were no differences in fungal levels between CCCs with respect to their dampness profile mainly due to high CCC ventilation rates. Since particle size is a factor that determines where a fungi particle deposits in the respiratory tract, this study provides useful information in the etiology of wheeze and rhinitis symptoms among the CCC attending children.



## 1. Introduction

Airborne fungal exposure may lead to allergic sensitization and symptoms of allergy and asthma (Bush and Portnoy, 2001; Stark et al., 2005; Park et al., 2006; IOM, 2000, 2004). There have been a large number of studies performed in temperate and cold climates, where indoor fungal genera/types and their concentrations in homes have been characterized and compared with corresponding outdoor levels (e.g. Shelton et al., 2002; Hargreaves et al., 2003). However, the quality of indoor air in child care centers (CCCs) has become an important issue during the last decade because of the increasing number of attending children (Smith, 2002; Monthly Digest of Statistics, 2005). Surprisingly, given the magnitude of the CCC population and the considerable amount of time children spend within them, information linking fungi concentration exposures within CCCs and health is sparse in the tropics.

Concentrations of indoor airborne fungi are mainly characterized by their generation within, their concentration outside and the rate of air exchange (AER). Indoors, fungi can be potentially dispersed via occupant activities such as cooking, resuspension and cleaning activities with significant contributions generated by the presence of dampness (Lehtonen et al., 1993; Meklin et al., 2002a; Zuraimi and Tham, 2008). But, the air exchange rates of CCCs play a role in either increasing or decreasing fungal exposure indoors. Surveys have reported that lower ventilation is associated with increased concentrations of fungi when indoor sources are present (Meklin et al., 2002a; Zuraimi and Tham, 2008) while other studies have shown that outdoor fungi spores are a major source for indoor levels especially for naturally ventilated buildings with high AERs (Reponen et al., 1994; Burge, 2002; Zuraimi and Tham, 2008). The proximity of vegetation such as parks to a building can also increase fungal concentration levels under the latter condition (Hargreaves et al., 2003).

Literature reviews have linked the presence of dampness with asthma and allergies (Bornehag et al., 2001; IOM, 2000, 2004). Indeed, studies performed in the cold Nordic climates have shown

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that fungal concentrations in moisture-damaged CCCs are higher than in non-damaged ones (Reponen et al., 1994; Meklin et al., 2002a,b). Concomitantly, aerobiological surveys in Singapore have documented abundance of outdoor fungal spores year round (Lim et al., 1998; Ong, 2004). Interestingly, the survey data have suggested that the trends for acute asthma exacerbation were associated with variations in the outdoor airspora profile (Lee et al., 1994; Chew et al., 1998) even though indoor fungi can grow with ease on building material surfaces in the tropics (Lim et al., 1989).

Currently, little is known about the impact of exposures to different fungi constituents in exacerbating adverse asthma and allergic symptoms (IOM, 2004; Nevalainen and Seuri, 2005) especially in the tropics (Ong, 2004). An important exposure parameter that may link asthma and allergic outcomes to fungi exposures could be the particle size. Smaller sized particles can penetrate deeper into conducting airways of the lungs, and some have been found to produce inflammatory effects on the respiratory mucosa of allergic asthmatics, as demonstrated in bronchoalveolar lavage studies (Metzger et al., 1986). Further, experimental studies demonstrated that fraction of particles deposited in the bronchial tree is inefficiently cleared where deposited amount increase with decreasing particle geometric diameter (Smith et al., 2008). Verily, very few field studies relate fungi particle sizes with allergic outcomes.

As part of a larger epidemiologic study on CCC exposures and asthma, allergies and respiratory symptoms among attending children (Tham et al., 2008), we investigated the differences in culturable fungi characteristics in CCCs with low and high prevalences of wheeze and rhinitis. Fungal characteristics studied include total as well as fungi genera/types and their aerodynamic particle sizes. We also evaluated if indoor dampness and proximity to parks are associated with these differences.

## 2. Methods

### 2.1. Study design

The sampling population for this work was obtained from the cross-sectional study performed on 104 randomly selected CCCs in Singapore (Tham et al., 2008). An International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire distributed to all parents of children in the CCC was used to establish the prevalence of wheeze and rhinitis. Wheeze was defined by the presence of wheezing or whistling in the chest within the last year while rhinitis was defined as problem with sneezing or a runny or blocked nose when he/she did not have a cold or flu within the last year (Asher et al., 1995). A total of 77 CCCs selected fulfilled the criteria to have at least 30 respondents to the questionnaire and a minimum of 50% response rate. The symptoms' prevalences of each CCC were then ranked from the lowest to highest (prevalence range of wheeze: 5.2–34.4%; prevalence range of rhinitis: 11.4–46.8%) (Fig. 1).

For each symptom, the CCCs were subsequently divided into two prevalence groups for environmental sampling: low and high prevalence groups. For wheeze symptom, there were 10 CCCs from the low and high wheeze prevalence groups each while for rhinitis symptom, there were 9 CCCs from the low and high rhinitis prevalence groups each. In total, 28 CCCs were included for environmental sampling since 5 CCCs belong to high prevalence groups from both wheeze and rhinitis symptoms and 5 belong to low prevalence groups from both symptoms. There was no CCC belonging to both high prevalence wheeze and low prevalence rhinitis groups or low prevalence wheeze and high prevalence rhinitis groups. The median (range) wheeze prevalence for the low and high prevalence groups was 6.0% (5.2–7.9%) and 26.7% (24.6–34.4%) respectively. The median (range) rhinitis prevalence for the low and high prevalence groups was 13.9% (11.4–17.6%) and 39.2% (35.7–48.6%) respectively. When the low wheeze/rhinitis prevalence groups were tested ($\chi^2$ test) against the high wheeze/rhinitis prevalence group, significant differences between them were found ($p < 0.001$). Details of the selection of high and low prevalence CCC group procedures were described elsewhere (Tham et al., 2008).

### 2.2. Air sampling

For each CCC, 2–4 indoor and 1 outdoor sampling locations were identified. Indoor samplings were performed in the middle of the classroom near the breathing zone of children (approximately 0.5–0.7 m). Samplings were conducted in the middle of the week and in the morning from 9 am to 11 am. Outdoor samplings were performed near the building not exceeding 5 m. The six-stage Anderson Sampler (Graseby-Andersen, Atlantis, GA, USA) (Andersen, 1958) collecting airborne fungus onto nutrient agar at an airflow rate of 28.3 L $min^{-1}$ for 2 min sampling intervals was employed. The sampler collects fungus on six stages according to their aerodynamic diameter (0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–7.0 and >7.0 μm) where 95% or more of the fungus within the size ranges can be collected on each stage (Andersen, 1958). Samples were collected in triplicates, using the same sampler sequentially. Sterile plastic disposable Petri dishes filled with Rose Bengal Agar (RBA, containing 0.5% streptomycin) (King et al., 1979) were used for collecting the air samples based on its ability to present both the concentration and the diversity of airborne fungi found in the tropical hot and humid climate. Pre-study comparison results using different media revealed RBA to provide the best results – in terms of the number of colonies and the range of fungal species showing up, and a more controlled fungal growth rate (which prevented smothering by the faster growing species). In total, the number of indoor and outdoor plates retrieved from 6-stage sampling amounted to 1818. After averaging the triplicates, there were 73 indoor locations and 28 outdoor locations.

After sampling, the RBA plates were incubated in the laboratory at room temperature (25 °C). After 4 days' incubation, fungal colonies on the plates were enumerated. Fungal concentrations in colony forming units (CFU) per cubic meter of air were calculated taking into account for positive hole corrections (Macher, 1989). The median precision of the triplicate samples calculated as percent relative standard deviation (%RSD) of the average concentration ranges from 17.6% (2.1–3.3 μm) to 100% (0.65–1.1 μm). Fungal identification begins after 7 days' incubations, either on the original sampling media-RBA plates, or on the sub-culture media-Potato Dextrose Agar (PDA) plates. With the aid of identification manuals (Barnett, 1960; Ellis, 1971, 1976; Ellis and Ellis, 1990), airborne culturable fungi were identified by genus level based on morphological characteristics of conidia and conidiophores. Microscopic inspection is performed with 10× and 40× objective magnifications using an optical microscope. Culturable fungi failing to produce spores after 30 days' incubations were labeled as sterile mycelia. For each sample, the geometric mean diameters of culturable fungal particles were first calculated ($D_g$). Subsequently, the average geometric mean diameters ($D_{g,\ ave}$) and their geometric standard deviations (GSDs) for different CCC types were determined (Hinds, 1999).

For each of the sampling locations, temperature, relative humidity, air velocity and air exchange rates (AERs) were sampled simultaneously. Indoor air temperature and relative humidity data were collected continuously by HOBO H8 Family data loggers (Onset Corporation, Bourne, MA, USA). The HOBO loggers were