Atmospheric Environment 128 (2016) 268–275

Contents lists available at ScienceDirect

Atmospheric Environment

journal homepage: www.elsevier.com/locate/atmosenv

Distribution of bacteria in inhalable particles and its implications for health risks in kindergarten children in Hong Kong

Wenjing Deng [a, *], Yemao Chai [a], Huiying Lin [a], Winnie W.M. So [a], K.W.K. Ho [a], A.K.Y. Tsui [b], R.K.S. Wong [b]

[a] *Department of Science and Environmental Studies, Centre for Education in Environmental Sustainability, The Hong Kong Institute of Education, Tai Po, NT, Hong Kong*

[b] *Department of Early Childhood Education, The Hong Kong Institute of Education, Tai Po, NT, Hong Kong*

HIGHLIGHTS

- The bacterial distribution and concentration in $PM_{2.5}$ in kindergartens are detected with a molecular biology method.
- Inhalable bacteria genera are compared in indoor and outdoor samples.
- Health risks from airborne bacteria to kindergarten children are analyzed.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form
8 January 2016
Accepted 8 January 2016
Available online 11 January 2016

Keywords:
Bacteria
Inhalable particles
Health risks
Kindergarten children

ABSTRACT

Health risks in children caused by bacteria in indoor environments have attracted much attention in recent years. There are many harmful bacteria, and children have greater health risks than adults in the same environment. To investigate the association between children's health risks and the distribution and concentration of bacteria in particulate matter smaller than 2.5 μm in indoor and outdoor air at three kindergartens in Hong Kong, quantitative polymerase chain reaction was used to determine the concentration of bacteria, and the terminal restriction fragment length polymorphism method targeting 16S ribosomal RNA genes was used to predict the phylogenetic airborne bacterial community structures. The bacterial concentrations indoors were higher than those outdoors in the three kindergartens, but no obvious differences were found ($P > 0.05$). Statistical analysis showed that the different schools had significantly different concentrations ($P < 0.05$). The abundance of bacteria in schools downtown (in Kowloon) was the greatest, reaching 3.3×10^3 to 4.95×10^4 cells per m^3. However, the results showed no significant differences between the microbial populations obtained indoors and those obtained outdoors. The dominant genera were very similar among the six samples. Our results suggest that the majority of the inhalable bacteria were harmless to humans. Only a small fraction of microbial pathogens were identified, and their relative abundance appeared to increase as the concentration of particulate matter pollution increased. Analysis of these bacteria can give important clues regarding the exposure of kindergarten children to bacteria in indoor and outdoor air.

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* Corresponding author.
E-mail address: wdeng@ied.edu.hk (W. Deng).

http://dx.doi.org/10.1016/j.atmosenv.2016.01.017

1. Introduction

Indoor air pollution is a rising issue in both developed and developing countries. Airborne particulate matter (PM), especially that smaller than 2.5 μm ($PM_{2.5}$), is a complex and dynamic mixture of components with chemical, physical, and biological origins and different chemical and physical properties (Putaud et al., 2004). In the past decade, many research projects have investigated the risks that PM poses to human health and to ecosystems (Samet et al., 2006), but these studies have typically focused on nonbiological particles (Colbeck and Lazaridis, 2009). In recent years, research interest has grown concerning the biological fraction of PM (bioaerosols) (Jones and Harrison, 2004), and an increasing body of evidence suggests that the biological fraction may play a critical role in the effects of PM on human health and on biological systems (D'Amato et al., 2002; Jaenicke, 2005). Lee et al. (2006) reported that bioaerosol sources are often located outdoors, therefore, the influence of these sources on human exposure largely depends on the fraction of outdoor bioaerosol, which is subjected to indoor penetration. However, the bacteria in indoor air can originate from the outdoor environment or from anthropogenic sources such as the building's occupants and human activities. Many people spend more than 90% of their time in an indoor environment (Klepeis et al., 2001). Because children spend a considerable amount of time in kindergarten (approximately 40 h per week), it is extremely important to ensure that conditions are appropriate and advantageous for their health and development. Recently, there has been great concern about the potential health hazards of biological components in airborne PM, especially about the levels of allergenic or toxigenic bacteria and their association with indoor air quality (Zucker and Muller, 2004; Liao et al., 2010; Hsu et al., 2011). Gram-negative bacteria that produce endotoxins possess strong allergens and are associated with respiratory diseases. Endotoxins can cause acute toxic effects, including fever, malaise, and asthma (Di Giorgio et al., 1995). In Singapore, an increase in the mortality rate from asthma was observed in children 5–14 years of age from 0.21 per 100,000 persons in the 1970s to 0.72 per 100,000 persons in the 1990s (Ng and Tan, 1999). This rising trend has also been reported in Hong Kong (So et al., 1990). The prevalence of asthma has increased in Hong Kong over the past two or three decades, especially in children.

Given their age and susceptibility, young children may be more likely than adults to suffer from the potential health hazards of airborne bacteria (Kahan et al., 2005). Pathogenic bacteria in the air are of particular concern as potential causes of disease in children. However, no previous studies have investigated the indoor biological contaminants of inhalable $PM_{2.5}$, especially in sensitive groups such as young children 2–6 years of age. Several studies have been carried out on indoor and outdoor airborne bacteria using traditional culture-based methods (Fang et al., 2007; Kim and Kim, 2007; Zhu et al., 2003). The results of several of these studies showed the diversity of the community of indoor airborne bacteria in a kindergarten, but the culture-based methods constrained the identification to a limited number of cultivable species. Thus, we aimed to use a molecular method to detect the concentration and community distribution of both cultivable and non-cultivable bacteria in inhalable $PM_{2.5}$ samples that affect indoor air quality and children's health in kindergartens in Hong Kong.

With the development of modern molecular biotechnologies, a variety of molecular approaches were developed and used to study bacterial diversity. Among these tools, terminal restriction fragment length polymorphism (T-RFLP) method was seen as a rapid, moderate-cost and high throughput molecular tool for assessing bacterial community structure (Païssé et al., 2010). T-RFLP used restriction site polymorphisms in PCR-amplified rRNA genes to produce fluorescently labeled terminal restriction fragments that differ in length (Liu et al., 1997). However, the use of this molecular method for phylogenetic identification still limited. Thereby, many webs based tools like TORAST (http://www.torast.de), TAP-TRFLP (http://www.rdp.cme.msu.edu) and The Microbial Community Analysis (MiCA) website (http://mica.ibest.uidaho.edu/.) have been shown which allowed to predict T-RFs from 16S rRNA gene sequences in the database based on user input of PCR primers and restriction enzymes and allowed to compare fragments obtained from T-RFLP analysis to the fragment sizes predicted from known 16S rRNA gene sequences (Belila et al., 2012). In addition, real-time quantitative PCR (Q-PCR) – another molecular technique is now widely applied in environmental research and is also used to measure total airborne bacteria in varied environments based on the 16S rRNA gene (Oppliger et al., 2008).

In this work, terminal restriction fragment length polymorphism (T-RFLP) technology via MiCA database and quantitative polymerase chain reaction (Q-PCR) amplified with universal primers (EUBf933 and EUBr1387) were used to provide a thorough description of the airborne microbial communities in kindergartens. Samples were taken from inside and outside three kindergartens in different locations to describe the variations in the bacterial community structure and composition and their health effects on the children.

2. Materials and methods

2.1. Description of sampling sites

Studies were conducted at three kindergartens located in the three different areas of Hong Kong, i.e., the New Territories (K1), Kowloon (K2), and Hong Kong Island (K3) as shown in Fig. 1. The kindergartens were in service between 8.00 am and 5:30 pm. The building's age, construction, and size and the number of students and staff varied among the kindergartens. Each kindergarten was equipped with central air conditioning, and ventilation was provided naturally through the windows. The sizes of the rooms in which sampling were carried out varied, and each classroom was occupied by an average of 30 children for much of the day. K1 lies at the back of a residential building near a large park, and six rooms are used for teaching. K2 is a larger kindergarten situated around the business district; its main building has two floors that include nine classrooms and several offices. In addition, several buildings were being constructed in the area around the kindergarten. K3 is located in a very large community with a more expansive green area and has considerably fewer children than the other kindergartens.

2.2. Sample collection

Samples were collected on three occasions at each of three kindergartens for young children (2–6 years of age) in Hong Kong. Six samples including indoor and outdoor air were collected at the same time and were labeled KI1 to KI3 and KO1 to KO3, respectively. $PM_{2.5}$ was collected at each school during study. The selection of schools was based on their locations in different microenvironments and their ventilation types to ensure that a general view of the exposure to the contaminants in the area was obtained. The samples were collected 95–120 cm above the floor at each site, similar to the height of the children. Ambient air was taken at an average flow rate of 5 L/min with a Mini Vol™ air sampler (Airmetrics, USA). Particulate-associated bacteria were isolated from the atmosphere by drawing air through a Whatman quartz 47-mm fiber filter for approximately 24 h. Blank control samples were collected by placing a sterilized filter inside the