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Characteristics of airborne bacteria in Mumbai urban environment

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HIGHLIGHTS

- Study demonstrated association between endotoxin and PM induced pro-inflammatory response
- Human or animal flora are identified as vital component of airborne culturable bacteria in Mumbai
- Study shows significant presence of pathogenic and opportunistic bacteria in the study area
- Study highlights the importance of airborne biological particles of ambient PM

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ABSTRACT

Components of biological origin constitute small but a significant proportion of the ambient airborne particulate matter (PM). However, their diversity and role in proinflammatory responses of PM are not well understood. The present study characterizes airborne bacterial species diversity in Mumbai City and elucidates the role of bacterial endotoxin in PM induced proinflammatory response in ex vivo. Airborne bacteria and endotoxin samples were collected during April–May 2010 in Mumbai using six stage microbial impactor and biosampler. The culturable bacterial species concentration was measured and factors influencing the composition were identified by principal component analysis (PCA). The biosampler samples were used to stimulate immune cells in whole blood assay. A total of 28 species belonging to 17 genera were identified. Gram positive and spore forming groups of bacteria dominated the airborne culturable bacterial concentration. The study indicated the dominance of spore forming and human or animal flora derived pathogenic/opportunistic bacteria in the ambient air environment. Pathogenic and opportunistic species of bacteria were also present in the samples. TNF-α induction by PM was reduced (35%) by polymyxin B pretreatment and this result was corroborated with the results of blocking endotoxin receptor cluster differentiation (CD14). The study highlights the importance of airborne biological particles and suggests need of further studies on biological characterization of ambient PM.

1. Introduction

Airborne particulate matter (PM) contain significant fraction (16%) of biological components (Jaenicke, 2005) and likely to play a significant role in health and climate. Airborne bacteria are one of the important components of airborne biological particles in natural and urban environment (Jaenicke, 2005; Brodie et al., 2007; Elbert et al., 2007; Jaenicke et al., 2007; Bowers et al., 2011). Bacteria present in the ambient atmosphere originate from natural and anthropogenic sources such as plants, animals, soil, water bodies, waste dumping ground, wastewater treatment plants and agricultural activities. Many human activities such as solid waste and sewage transport, processing and conducive humid environment may enhance the abundance of culturable airborne bacteria in an urban environment.

Properties of urban airborne bacteria are studied over decades (Mancinelli and Shulls, 1978; Brodie et al., 2007; Fang et al., 2007; Bowers et al., 2011). These studies identified the dominant bacterial species in urban environments and have shown that the species properties and their proportions vary significantly among urban settings. Change in proportion of dominant airborne bacterial species with meteorological and micro-environmental properties was studied in various environmental settings (Lighthart et al., 2009; Wang et al., 2010). However, important factors leading to the variability in species composition, source contribution and survivability in urban air environment are not yet well understood. Health impacts of airborne bacteria and their components are also not well studied.

Endotoxin is a cell wall component of Gram-negative bacteria (GNB). Initially, significant levels of airborne endotoxin was detected in several occupational environments such as cotton industry, agriculture processing, barns, sewage treatment, composting and waste handling. These studies have shown that in occupational environments, endotoxin is of one the most important component associated with

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health effects of PM exposure. Similarly, endotoxin in indoor environments such as in homes and offices has been characterized and linked to adverse health effects. Endotoxin is known to be present in ambient PM at low levels. Now it is known that ambient airborne endotoxin plays a significant role in particulate matter induced proinflammatory response. However, the exact role of low levels of endotoxin in inducing proinflammatory response is not well understood (Gangamma, 2012a, 2012b).

Mumbai is one of the most densely populated cities in the world. Many human activities such as solid waste and sewage transport and processing may enhance the ambient concentration of bioaerosols in the city. However, the characterization of biological components of the ambient PM in the city has not been previously attempted. The present study characterizes species diversity of airborne bacteria in Mumbai. The paper adopts two methods to highlight the importance of airborne bioparticles in Mumbai. First method utilizes the species characterization information of airborne bacteria. Second method utilizes endotoxin which is an important fraction of airborne bioparticles to explain the inflammatory response of total particulate matter. Based on these methods, the study accentuates the importance of biological fraction of urban PM in Mumbai.

2. Materials and methods

2.1. Sampling of airborne bacteria and endotoxin

The air samples were collected from the four ambient air quality monitoring sites (Fig. S1) in Mumbai during April–May 2010 with microbial Anderson impactor (Thermo Scientific USA, n = 22) and biosamplers (SKC USA, n = 34). The study planning and methods are given in the supplement. The bacterial colonies were separated from each impactor sample (n = 22) and were identified further to their species level (Gangamma et al., 2011) with Biolog Manual System-1 (Biolog, Inc., USA).

2.2. Whole blood assay for determination of TNF-α production

Twenty randomly selected biosampler samples were lyophilized, and made up to 600 μL with endotoxin free water. The sample aliquots were stored at −20 °C for further analysis. The aliquots were subjected to the whole blood (ex vivo) assay for measuring the tumor necrosis factor (TNF-α). Venous blood was collected in EDTA coated vacutainer (BD Bioscience, India) from six healthy donors. The blood samples from donors were pooled and used within 2 h of withdrawal. The assay was conducted in a single batch. The endotoxin content of donor's blood sample was measured and was found to be below the detection limit of the assay. Details of methods are explained elsewhere (Gangamma et al., 2011). Briefly, 100 μL of fresh blood was incubated with 100 μL of the samples and 350 μL of 0.9% saline in a pyrogen free tube at 37 °C for 16 h. The cell free supernatant was used to measure the TNF-α produced by the cells as per the manufacturer's instruction (BD Bioscience, India). Samples of field blank were also incorporated in the ex vivo. No detectable amount of TNF-α was observed from the blanks. All samples and controls were analyzed in duplicate.

2.3. Endotoxin removal using polymyxin B sulfate

Endotoxin is a stable and immune-sensitive component of Gram negative bacteria (GNB) and is a reasonable surrogate for bacteria in the inflammatory response assessment. The biosampler samples were measured for endotoxin content using kinetic limulus ambeocyte lysate (LAL) assay. Further the samples were treated with polymyxin B sulfate coated agarose beads (10 μg/mL; Sigma-Aldrich, St Louis) for 30 min to neutralize the endotoxin (Gangamma et al., 2011). The supernatant was collected and endotoxin concentration was measured again using LAL assay. The samples were subjected to ex vivo and TNF-α was determined as described above. The assay was conducted in a single batch. The specificity of polymyxin B to endotoxin was evaluated using recombinant interferon-gamma (rIFN-γ) and bacterial surface components (BSC) of Gram positive bacteria (GBP). Details of specificity test are discussed in the Supplementary material. No significant induction of TNF-α was observed after treatment with polymyxin B (Fig. S2).

2.4. Blocking of endotoxin receptors and TNF-α response

The detailed experimental protocol is described in supplement. In brief, 100 μL of blood cells were incubated for 1 h with 20 μL of anti-CD14 (BD Bioscience, India) prior to the ex vivo assay. The supernatant was collected for measurement of TNF-α. The assay with and without anti CD14 was conducted in single batch.

2.5. Statistical analysis

Mann–Whitney-U (MWU) (Conover, 1971) test was used to verify the significant difference in the induction of TNF-α by air samples and air samples treated with polymyxin B. Principal component analysis (PCA) was performed for the identification of bacterial species groups (components) and their relative dominance in the samples (factor scores). Varimax rotation was used to improve the difference between the principal components. A program in FORTRAN was written for performing the above analysis (Reymend and Joreskog, 1996). Regression analysis was used to find the significance of the linear relationship between two variables.

3. Results

3.1. Spore forming bacterial species dominates airborne bacteria

Gram positive bacteria were found to be predominantly present across all the sampling sites and represent about 82% of total culturable bacteria (Fig. 1). A total of 28 species belonging to 17 genera were identified (Table S1). The results show that the spore forming groups of bacteria (SFB) such as *Bacillus* species dominate the total culturable bacterial concentration. The SFB correlates well with the total concentration of bacteria ($R^2 = 0.884$, $p < 0.001$) and may indicate the requirement of spore forming property for bacterial species to survive in the harsh air environment.

To understand further characteristics of airborne bacterial species, principal component analysis (PCA) for the observed species concentration was carried out. The analysis shows that the total variability in species abundance among the samples can be significantly captured by three eigen factors (82%). Data variability explanation by three eigen factors suggests the existence of a few common characteristics, which

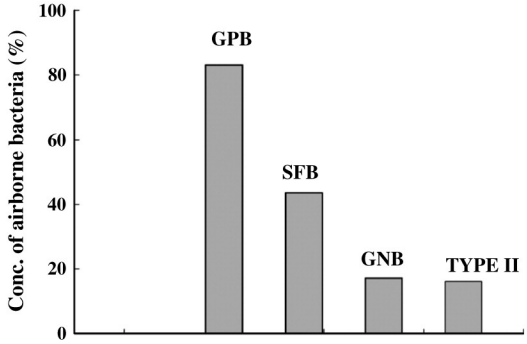

Fig. 1. Colonies collected in the Anderson impactor were isolated and the species were characterized. The fraction of Gram negative bacteria (GNB), Gram positive bacteria (GPB), spore forming bacteria (SFB) and Type II bacterial species are plotted in the figure. The GPB and SFB dominated the total identified culturable bacterial species. 16% of identified bacterial species belongs to Type II.