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'Core species' in three sources of indoor air belonging to the human micro-environment to the exclusion of outdoor air

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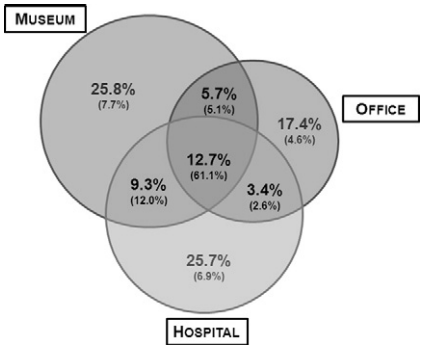
HIGHLIGHTS

- The enclosed spaces revealed a specific common bacterial signature for their air.
- 'Core species' signing the anthropogenic indoor air were identified.
- Man should be considered as the main factor generating the microflora indoor air.
- A better knowledge of the microbiological content of indoor air is needed.
- It will improve our future ability to manage indoor air quality.

GRAPHICAL ABSTRACT

Sequences were grouped into OTUs at a 97% level of sequence similarity. OTUs overlapping between the museum, office and hospital are shown in the inset. Sequence abundances are given in brackets.



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ABSTRACT

Although we spend the majority of our lives indoors, the airborne microbial content of enclosed spaces still remains inadequately described. The objective of this study was to characterize the bacterial diversity of indoor air in three different enclosed spaces with three levels of occupancy, and, in particular, to highlight the 'core' species, the opportunistic pathogens and their origins. Our findings provide an overall description of bacterial diversity in these indoor environments. Data gathered from the three enclosed spaces revealed the presence of a common indoor signature (60% of total sequences in common). This work will provide a clearer understanding of the dominant groups of bacteria encountered in enclosed spaces: *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. Thus, certain evidence revealed a connection between 'core' species and the human micro-environment (20% of phylotypes and 12% of sequences of human origin). Overall PCA analysis showed that the indoor environment is influenced mainly by the microbial diversity from nose and skin. Among the 'core species' found during this study, a large number (72% of all pathogen-related sequences were concentrated in 'core species') of genera and species are known to be responsible for opportunistic or nosocomial diseases or to include human commensal bacteria such as *Mycobacterium* sp., *Acinetobacter baumanii*, *Aerococcus viridians*, *Thermoactinomyces vulgaris* or *Clostridium perfringens*.

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1. Introduction

Which element do human beings most widely share? Not food, not water but air. This unavoidable sharing, a potential vector for many diseases, has increased exponentially in recent years due to changes in our lifestyle. Roughly indoor air accounts for 0.3 millionth of tropospheric air. The Earth's population continues to grow, people travel more, but 50% of them live in urban areas and spend up to 90% of their lifetime indoors (children in day care, nursing homes, dwellings, offices, transport systems or public buildings) (Höppe and Martinac, 1998). Those facts underlie the importance of assessing the risks associated with micro-organisms in enclosed spaces. Despite general concern with public health, the microbial content of indoor air, along with the role of air in spreading pathogens, remains poorly described in comparison to that of other environments such as outdoor air, soil, food or water. Today, indoor air scientific field remains largely unexplored. And yet the transmission of infectious agents cannot always be avoided because controlling the dissemination of airborne pathogens is far more difficult than preventing the pathogenic contamination of surfaces, water or food.

Most studies of enclosed spaces have linked the indoor environment with the outdoors, entailing a description of both settings. But some studies have presented indoor air quality as being closely linked to major sources indoors, including human activity or mere occupation (Kotzias et al., 2009; Wichmann et al., 2010; Zuraimi and Tham, 2008). Moreover, physical activities in an indoor space cause the resuspension of sedimented particles from indoor floor dust (Almeida et al., 2011; Fromme et al., 2007, 2008). Zuraimi and Tham (2008) showed that higher levels of human-related bacteria were associated with high occupancy rates, irregular floors and the frequency of surface cleaning. Liu et al. (2000) investigated bacterial concentrations in the indoor air of two elementary schools and hypothesized that the children and teachers might be the principal source of bacterial contamination. Furthermore, a comparison between airborne micro-organisms found in shopping centres and those in other environments located nearby indicated that indoor-air micro-organisms seemed to arise from indoor domestic niches (Tringe et al., 2008). Thus, direct human emissions and indirect dust emissions appear to be significant sources of airborne bacteria in the indoor environment (Hospodsky et al., 2012; Kelley and Gilbert, 2013; Korves et al., 2013; Meadow et al., 2013; Qian et al., 2012).

Because airborne micro-organisms are present at low concentrations, their collection is a challenging task. Collection systems with high flow rates may be necessary to permit molecular analysis under optimum conditions (Gaüzère et al., 2013). Sampling larger volumes of air using integrated systems may also be an efficient alternative (Tringe et al., 2008). Although the diversity of cultivable bacteria in confined spaces is relatively well-documented, most studies employ culture methods that do not fully describe microbial diversity. Indeed, not quite 1% of environmental micro-organisms only can be cultivated (Amann et al., 1995) and microbial aerosols appear to be particularly recalcitrant on account of the constraints hampering the methods used for their collection (Radosevich et al., 2002; Wang et al., 2001). Recently, however, the investigation of microbial diversity in indoor air using molecular methods has developed considerably (Angenent et al., 2005; Osman et al., 2008; Tringe et al., 2008). Some studies have successfully applied pyrosequencing in talking various questions about indoor-air microbial communities (Gaüzère et al., 2014; Hospodsky et al., 2012; Kembel et al., 2012; Meadow et al., 2013). The data obtained have demonstrated the presence of all microbial domains (*Bacteria*, *Eukarya* and *Archaea*). Examination of these data reveals a bacterial diversity which differs from that observed using culture methods: bacterial diversity is largely dominated by *Alpha-*, *Beta-* and *Gamma-Proteobacteria*. There appears to be a diversity specific to indoor air. In terms of the health risks associated with the presence of pathogenic species in air, these studies have highlighted the over-representation of opportunistic pathogen species such as *Propionibacterium acnes*, *Staphylococcus* spp., *Streptococcus* spp. or *Stenotrophomonas maltophila*. The pathogenic species found in air are often associated with nosocomial infections (*Acinetobacter baumanii*, *Clostridium jejeikieum*, etc.) and respiratory infections (*Mycobacterium* spp.).

There is currently a lack of molecular data both on the environmental airborne species found in enclosed spaces and on the environmental origin of the sequences present.

In this overall context, the aim of this study was to characterize the bacterial diversity of indoor air and, in particular, to highlight 'core species', opportunistic pathogens as well as the origin of indoor bacteria found in three different enclosed spaces with three different degrees of occupancy (low: office, high: museum and average: hospital).

2. Materials and methods

2.1. Measurement sites

The measurement of the indoor bioaerosols was done in France during four-week sampling periods (28 days) in 2010: 14 January–11 February in Lagny Hospital (H), 16 July–13 August in an office in Champs-sur-Marne (O) and 30 September–28 October in the Louvre Museum in Paris (M).

These three sites differ in terms of their uses and degrees of occupancy (on average: 10,000 people in the museum (M), 1000 people in the Emergency Paediatric Department of the hospital (H) and 20 people in the office (O)).

Only the entrance and the waiting room of the Emergency Paediatric Department in the hospital were investigated. This site is considered to be highly sensitive to the presence of pathogens. In the Louvre Museum, measurements were carried out on the second floor of the Richelieu wing, which represents a huge sampling volume. The office was an open-plan area occupied during working hours (5 days a week between 08:00 and 19:30).

2.2. Collection of samples

A sampling device was developed that could be connected to the existing ventilation system of the buildings (Fig. 1). This filtration device operated at a rate of 6.3 m^3/h. The duration of each sampling period was 4 weeks and an average volume of 4200 m^3 air was filtered. A cellulose high efficiency particulate air (HEPA) 13 filter (CAMFIL FARR) was used, its diameter 200 mm and its collection efficiency 99.95% for particles of 0.3 μm. The filters were then scraped into molecular-grade water.

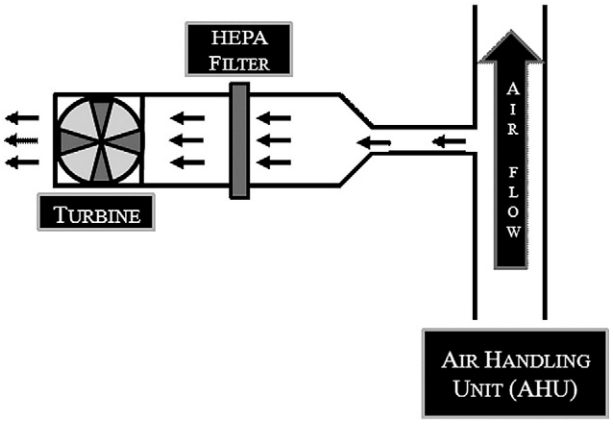

Fig. 1. A schematic view of the sampling filtration system used at the three sites.