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Filters from taxis air conditioning system: A tool to characterize driver's occupational exposure to bioburden?

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ABSTRACT

Bioburden proliferation in filters from air conditioning systems of taxis represents a possible source of occupational exposure. The aim of this study was to determine the occurrence of fungi and bacteria in filters from the air conditioning system of taxis used for patient transportation and to assess the exposure of drivers to bioburden. Filters from the air conditioning systems of 19 taxis and 28 personal vehicles (used as controls) operating in three Portuguese cities including the capital Lisbon, were collected during the winter season. The occurrence and significance of bioburden detected in the different vehicles are reported and discussed in terms of colony-forming units (CFU) per 1 m^2 of filter area and by the identification of the most frequently detected fungal isolates based on morphology. Azole-resistant mycobiota, fungal biomass, and molecular detection of *Aspergillus* species/strains were also determined. Bacterial growth was more prevalent in taxis (63.2%) than in personal vehicles (26.3%), whereas fungal growth was more prevalent in personal vehicles (53.6%) than in taxis (21.1–31.6%). Seven different azole-resistant species were identified in this study in 42.1% taxi filters. Levels of fungal biomass were above the detection limit in 63% taxi filters and in 75% personal vehicle filters. No toxigenic species were detected by molecular analysis in the assessed filters. The results obtained show that bioburden proliferation occurs widely in filters from the air conditioning systems of taxis, including the proliferation of azole-resistant fungal species, suggesting that filters should be replaced more frequently. The use of culture based-methods and molecular tools combined enabled an improved risk characterization in this setting.

1. Introduction

The use of taxis for non-emergency patient transportation is important as it allows ambulances, and other specially equipped vehicles, to be available for patients that require special medical care during transport (Syed et al., 2013). In addition, compared to other public transportation systems, the use of taxis is more comfortable for patients, while preventing healthy passengers to share a closed area with potentially infected ones. However, the taxi cabinet is a restricted and often shared space. Several reports indicate that both taxi drivers and passengers face a high risk of exposure to a mixture of biological and chemical agents (Jo and Lee, 2008; Knibbs and Morawska, 2012; Kumar et al., 1990; Brodzik et al., 2014; Stephenson et al., 2014; Wu et al., 2010) with possible damage to health.

Bioaerosols are usually defined as aerosolized particles of biological origin. Examples of bioaerosols in occupational environments include fungal and bacterial spores/cells, fungal hyphae, pollen, viruses and amoebae, and also their metabolites (Eduard et al., 2012; Oppliger, 2014). Different adverse health effects due to the exposure to bioaerosols in occupational environments have been reported, including infectious diseases, acute toxic effects, allergies and cancer. Respiratory symptoms and lung function impairment are the most broadly studied and possibly among the most important bioaerosol-associated health effects (Rylander and Jacobs, 1997; Subbarao et al., 2009; Fogelmark et al., 1994; Eduard et al., 2001; Douwes et al., 2003). The microbiological contamination of air inside a vehicle may contribute to

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diverse symptoms and diseases in humans (Srikanth et al., 2008). Of note, bioaerosols present in the air of taxis cabinet can be especially hazardous to immunocompromised individuals (Ross et al., 2000; Prakash et al., 2014).

In order to protect vehicle occupants, filters from the air conditioning system in vehicles are intended to retain airborne bioburden. However, under favorable conditions, such as long periods of high relative humidity (> 80% R.H.), the bioburden proliferation on air filters and consequent release in the air stream to the vehicle cabinet represents a potential source of exposure to bioaerosols, especially if part of the respirable fraction (< 1.1 μm). Moreover, when the air conditioning system is turned on, the air stream passing through the filtration system could reaerosolize the bioburden and consequently carry it into the vehicle interior (Li et al., 2016) increasing the exposure. Greater exposure should be expected for taxi drivers as taxis are their workplace, with regular work shifts of 8 h a day 5 days a week accounting for considerably more time spent inside the vehicles than the passengers (O'Donoghue et al., 2007; Som et al., 2007; Jo and Yu, 2001; Nowakowicz-Dębek et al., 2017). Occupational exposure to bioaerosols and consequent health problems suffered by taxi drivers during patient transportation should be, therefore, acknowledged and prevented (Nowakowicz-Dębek et al., 2017; Walser et al., 2015).

The assessment of occupational exposure to bioaerosols by active air sampling has been described in vehicles of different types and uses, mainly in buses (Prakash et al., 2014; Nowakowicz-Dębek et al., 2017; Luksamijarulkul et al., 2004, 2005; Wang, 2011; Wang et al., 2013) and personal vehicles (Jo and Lee, 2008; Vonberg et al., 2010). Recently, we have witnessed an increased awareness about the potential of passive sampling methods to overcome the limitations of active methods (Viegas et al., 2015, 2017b), such as the reports on the use of surface swabs in personal vehicles (Stephenson et al., 2014) and buses (Prakash et al., 2014), settled culture media plates (Kumar et al., 1990), and air conditioning filters from personal vehicles (Li et al., 2016; Simmons et al., 1999; Diekmann et al., 2013). An important difference between active and passive sampling methods is that air samples collected by active methods reflect the load from a short period of time (mostly minutes), whereas passive methods can collect contamination from a larger period of time (weeks to several months) (Viegas et al., 2015, 2017b). The same trend has been applied in indoor air quality (IAQ) assessments to recover the bioburden from heating ventilation and air conditioning (HVAC) filters in buildings (Pang and Mu, 2007; Goyal et al., 2011; Noris et al., 2011). However, limited attention has been given to occupational exposure assessments to bioaerosols.

The complementarity of culture based-methods and molecular tools for the characterization of fungal burden in different occupational environments has been reported (Viegas et al., 2016b, 2016c; Degois et al., 2017) as being necessary for a complete and accurate risk characterization in workplaces (Viegas et al., 2017a). A recent work from our group describes the use of air filters to characterize the occupational exposure to bioburden of workers in fork lifters (Viegas et al., 2017b). Moreover, the increased occurrence of opportunistic fungal infections in immunocompromised patients, and the emergence of antifungal resistance, namely of *Aspergillus* sp., both in the clinical and in the environment (Fairlamb et al., 2016; Nature Microbiology, 2017) emphasize the importance of addressing the prevalence of antifungal resistance and the molecular detection of target species in the assessments of occupational exposure to fungal burden (Viegas et al., 2016a, 2017a).

Thus, although the assessment of occupational exposure to chemical agents in taxis drivers has been reported (Pang and Mu, 2007; Knibbs et al., 2010; Janicka et al., 2011; Lewné et al., 2006; Son et al., 2004; Miller-Schulze et al., 2010), to our knowledge, this is the first study that evaluates the occupational exposure of taxi drivers to bioburden through the evaluation of filters from the air conditioning system of taxis used for patient transportation, by culture-based methods, molecular tools, and screening of azole-resistance.

2. Materials and methods

2.1. Filters collection and characteristics

Nineteen taxis used for patients' transportation and 28 personal vehicles (used as controls) were prospected in three different cities around Lisbon (Lisbon, Loures and Setúbal) between January and March 2017 (winter season, temperatures ranging 10 °C). Sampling was performed by removing the filter from the air conditioning system in each vehicle. Filters were cut into three pieces of 2 cm^2 each (1.4 × 1.4 cm) and kept refrigerated at 4 °C before analysis.

Taxi filters were composed by activated charcoal and belonged to category 2 (typically ≥ 3.0 μm pores), in compliance with the protection requirements (EN 15,695) that ensure protection against dust inside the cabinet. Filters were used for a maximum of 15,000 kms, according to the preventive maintenance program in the taxi company. The ventilation provided for each taxi cabinet was one cabinet volume/minute. The criteria for filter replacement in the air conditioning system was the frequency established by the car brand to avoid filter blocking, as established in the preventive maintenance program and supervised by the taxis company.

Personal vehicle filters presented the same technical characteristics and replacement criteria than taxi filters, except for additional control measures to the preventive maintenance program (specific to each vehicle brand and dependent on the owner).

Additionally, one filter was obtained with the same technical characteristics but without prior use.

2.2. Filter sampling

One piece of filter was washed with 10 mL of 0.1% Tween™ 80 saline solution (NaCl 0.9%) for 30 min at 250 rpm on an orbital laboratory shaker (Edmund Bühler SM-30, Hechingen, Germany) (Viegas et al., 2017b), diluted and seeded on eight media: four for microbial screening (2% malt extract agar (MEA) supplemented with 0.05 g/L chloramphenicol; dichloran-glycerol agar (DG18); tryptic soy agar (TSA) supplemented with 0.2% nystatin; violet red bile agar (VRBA)); and four for azole-resistance screening (one Saboraud agar; and three Saboraud agars supplemented, respectively, with 4 mg/L itraconazole, 1 mg/L voriconazole, and 0.5 mg/L posaconazole (Arendrup et al., 2013)). MEA, DG18 and Saboraud media supplemented with azole antifungals were incubated at 27 °C for 5–7 days, and TSA and VRBA media were incubated at 30 °C and 35 °C for 7 days, respectively.

For fungal biomass assay, a second filter piece was extracted with 5 mL of PCR grade water for 10 min at 2500 rpm on a Maxi Vortex orbital shaker. The washed suspension was centrifuged at 4200 × *g* for 20 min; the supernatant was discarded except for 200 μL in which the cell pellet was re-suspended. The DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA) according to the manufacturer's instructions.

For molecular detection of *Aspergillus* sections, a third filter piece followed the same extraction procedure. Ten milliliters of the washed suspension were centrifuged at 3500 × *g* for 30 min; the supernatant was discarded except for 200 μL in which the cell pellet was re-suspended. The DNA extraction was performed as described.

2.3. Bioburden characterization

Fungal and bacterial densities (colony forming units (CFU) per 1 m^2 of filter area) were determined on the different culture media. Fungal species were identified microscopically using tease mount or Scotch tape mount and lactophenol cotton blue mount procedures. Morphological identification was achieved through macro and microscopic characteristics, as noted by De Hoog et al. (2000).