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Non-culturable bioaerosols in indoor settings: Impact on health and molecular approaches for detection

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HIGHLIGHTS

- Non-culturable fraction of bioaerosols is important but still misunderstood.
- Airborne non-culturable agents can be hazardous for human respiratory health.
- Molecular tools allow the detection of airborne non-culturable microorganisms.
- Culture-dependent and independent tools should be used for bioaerosol studies.
- Several research needs relating to non-culturable agents must be addressed.

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ABSTRACT

Despite their significant impact on respiratory health, bioaerosols in indoor settings remain understudied and misunderstood. Culture techniques, predominantly used for bioaerosol characterisation in the past, allow for the recovery of only a small fraction of the real airborne microbial burden in indoor settings, given the inability of several microorganisms to grow on agar plates. However, with the development of new tools to detect non-culturable environmental microorganisms, the study of bioaerosols has advanced significantly. Most importantly, these techniques have revealed a more complex bioaerosol burden that also includes non-culturable microorganisms, such as archaea and viruses. Nevertheless, air quality specialists and consultants remain reluctant to adopt these new research-developed techniques, given that there are relatively few studies found in the literature, making it difficult to find a point of comparison. Furthermore, it is unclear as to how this new non-culturable data can be used to assess the impact of bioaerosol exposure on human health. This article reviews the literature that describes the non-culturable fraction of bioaerosols, focussing on bacteria, archaea and viruses, and examines its impact on bioaerosol-related diseases. It also outlines available molecular tools for the detection and quantification of these microorganisms and states various research needs in this field.

1. Introduction

Bioaerosols are airborne particles that contain one or more components of biological origin, typically microorganisms such as bacteria, archaea, fungi or viruses. Bioaerosols are an important transmission route for infectious and sensitization agents, inducing infectious and non-infectious diseases in both animals and humans. However, there is a substantial lack of information in the field of bioaerosol research in terms of what organisms or components may be found in different environments, how they become aerosolised, and their impacts on health. Most studies have focused on occupational bioaerosol-related hazards faced by workers who are continually exposed to highly contaminated environments.

Bioaerosols have traditionally been studied using culture methods (Duchaine et al., 1999, 2000; Dutil et al., 2009; Meriaux et al., 2006b), which allow scientists to recover only culturable organisms, using prescribed growth media and specific conditions. However, only a small proportion of the total bioaerosol burden in any given environment is culturable. There are two main

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explanations for this: firstly, there are many biological components that cannot be measured by culture, such as cellular fragments and components; and secondly, many organisms are not easily cultured or are rendered unculturable by air sampling or aerosolization processes (Amann et al., 1995; Peccia and Hernandez, 2006). Whilst these non-culturable agents may not be infectious, they can cause inflammation and sensitization (Girard et al., 2009; Glazer et al., 2007; Thorne et al., 2006), and they can exacerbate existing chronic respiratory diseases.

Over the past ten years, studies using molecular methods for the detection of airborne microorganisms have revealed that non-culturable microorganisms are considerable constituents of bioaerosols. The importance of the application of these methods was illustrated when they were used in the discovery of significant amounts of archaea present in the atmospheres of several working environments, in particular in agricultural settings (Blais Lecours et al., 2012; Just et al., 2013; Nehme et al., 2009). Archaea are fastidious organisms (Khelaifia et al., 2013; Wolfe, 2011), and consequently, traditional techniques had never identified them in bioaerosol studies. Newly described bioaerosol components such as these could be a factor in several bioaerosol-related diseases with unknown aetiologies (Eduard and Heederik, 1998). For example, chronic bronchitis is prevalent in people who work in swine barns (Sahlander et al., 2012), but the definitive cause has yet to be clearly identified. It is therefore important to employ several detection approaches, including non-culturable testing.

Although moulds are an important component of bioaerosols (Zukiewicz-Sobczak, 2013) and, in some cases, can be included in the non-culturable fraction of bioaerosols, they will not be discussed in this review. Their conidia and spore structures are designed to allow airborne spreading, and thus are well adapted for transport through air currents. Additionally, they are not as sensitive to aerosolization and sampling stresses as bacteria, archaea or viruses and are easier to detect by culture-dependent methods. Fungal spore counts and fungal fragment evaluations are often used for their detection and few molecular approaches have been developed for their detection in bioaerosols (Gorny, 2004; Lee and Liao, 2014; Simon and Duquenne, 2014).

This review focuses on non-culturable agents, including bacteria, archaea and viruses, which are found in bioaerosols of indoor environments, and their potential impact on human health. We describe the molecular tools that can be used to detect non-culturable agents and also highlight the importance of combining culture-dependant and culture-independent approaches for thorough bioaerosol characterization. This review also states some research needs that must be addressed in the future.

2. Bioaerosol-related diseases

2.1. The impacts of bioaerosols on human health

Bioaerosols can affect human health in many ways. When carrying and transmitting infectious microorganisms, bioaerosols may initiate an infection in the respiratory tract or other parts of the body (Roy and Milton, 2004; Roy and Reed, 2012). Well known examples of airborne respiratory diseases include tuberculosis (LoBue et al., 2010), the common cold (Jaakkola and Heinonen, 1995), influenza (Bridges et al., 2003), and legionellosis (Nguyen et al., 2006). Only viable airborne agents can cause infectious diseases. In addition, bioaerosols that contain non-culturable microorganisms or their fragments can lead to chronic or acute diseases (Bunger et al., 2004; Fogelmark et al., 1991; Shahan et al., 1994). These non-infectious bioaerosol components can have immunogenic potential that induce sensitization diseases if they are present in sufficiently high concentrations (Falkinham, 2003). It is well documented that humans who are exposed daily to these airborne particles can develop allergic and chronic inflammatory responses (Eduard, 1997; Poole and Romberger, 2012). The impact of the inhalation of bioaerosol components on human health depends on various factors such as their infectivity, airborne concentration, immunogenicity and particle size.

Both the upper and lower respiratory tracts are exposed to bioaerosols. Diseases typically manifest in a particular part of the respiratory tract. Viruses are the main cause of infections in the upper respiratory tract (sinusitis, pharyngitis) (Lopardo et al., 2012), including seasonal influenza and viruses that cause the common cold (rhinovirus, adenovirus, coronavirus) (Louie et al., 2005). Sensitization diseases in the upper airways include allergic rhinitis and sinusitis and can be caused by whole or fragmented organisms (allergens, endotoxins, peptidoglycan and bacterial DNA) (May et al., 2012). On the other hand, lower respiratory tract diseases (bronchitis and pneumonia) are predominantly caused by bacteria (e.g. *Legionella* spp, *Streptococcus* spp., *Haemophilus influenzae*) (Dasaraju and Liu, 1996), although avian influenza (H5N1) can also infect the lower respiratory tract, along with parainfluenza viruses and respiratory syncytial viruses (Hall, 2001). This is also the site of chronic diseases such as asthma (bronchus) (Sferrazza Papa et al., 2014), hypersensitivity pneumonitis (alveolar) (Takemura et al., 2008) and chronic obstructive pulmonary diseases (bronchus) (Bettoncelli et al., 2014), which can be caused by bacterial and fungal cells and spores (O'Connor et al., 2013), allergens, peptidoglycan, and endotoxins (Reynolds et al., 2013), which are present on non-culturable agents.

2.2. Bioaerosols-related diseases in indoor settings

Bioaerosols are ubiquitous, but they are especially present in indoor environments due to the lack of ventilation and dispersal mechanisms. Indoor settings that contain particularly high concentrations of bioaerosols pose a greater risk to human health than facilities harbouring low airborne biological contaminants (May et al., 2012). Certain indoor settings such as agricultural settings, schools and homes may allow the accumulation of high bioaerosol concentrations, given the density of potential bioaerosol sources (humans, animals, plants) and the lack of efficient ventilation or air exchange systems (Table 1). People exposed to such indoor conditions are thus at risk of developing a wide range of respiratory and non-respiratory diseases and conditions (ATS, 1998; Douwes et al., 2003; Kirkhorn and Garry, 2000; Linaker and Smedley, 2002; National Institute for Occupational Safety and Health, 2007; van Kampen et al., 2012). These diseases are often not attributed to specific etiologic agents since a wide variety of organic products (such as allergens) can cause similar symptoms, and it is difficult to identify a specific cause.

2.3. The difficulty to correlate a disease with a bioaerosol component

For several indoor settings, including industrial facilities, domestic environments and medical clinics, information on total airborne bacteria, total airborne archaea, and airborne viruses is incomplete (Table 1), which prevents an overall knowledge and understanding of the total biological burden of these facilities. It is likely that the unknown non-culturable fraction of bioaerosols, which can be found only by culture-independent methods, plays an unsuspected role in bioaerosol-related diseases. With a continuing increase in the frequency of studies characterizing the non-culturable fraction of bioaerosols, shown in Fig. 1, the role this fraction plays on human health will become clearer.

Moreover, the correlation between the presence of traditionally