DETECTION OF MICROBIAL MARKERS IN OCCUPATIONAL SETTINGS WITH HURRICANE-RELATED WATER DAMAGE

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Abstract - Water damage can lead to an increase in indoor growth of bacteria and mold. Indoor exposure to these microbes and its components (i.e. endotoxin and β -glucans) can cause respiratory illness such as allergic rhinitis, exacerbation of asthma, hypersensitivity pneumonitis, and respiratory infections. Currently, there is no federal standard for occupational levels of endotoxin and β-glucans in indoor environments. Although, several federal organizations published guidelines and action plans for indoor environmental and air quality. Therefore, the purpose of this study was to characterize the potential risk of endotoxin and β-glucans exposure in occupational buildings that sustained water-damage as a result of Hurricane María landfall in Puerto Rico. Indoor dust samples were collected in June 2019 from 13 university-building areas located in four different municipalities with varying degrees of water-damage. Horizontal (i.e. desks) and vertical (i.e. walls) surface sampling was conducted with sterile polyurethane swabs. Endotoxin and β-glucans concentrations were determined using the kinetic Limulus Amebocyte Lysate and Glucatell assays, respectively. Finally, to determine a potential risk of exposure we evaluated the concentrations of endotoxin and β -glucans from our study and compared it with other studies to determine if these microbial components represents a health risk for the workers.

Key words: Environmental exposure, occupational assessment, water damage, indoor environmental quality, microbial components.

Resumen - El daño causado por agua en ambientes interiores puede provocar un aumento en el crecimiento de bacterias y hongos. La exposición en interiores a estos microbios y sus componentes (endotoxinas y β-glucanos) puede causar enfermedades respiratorias como rinitis alérgica, exacerbación del asma, neumonitis por hipersensibilidad e infecciones respiratorias. Actualmente, no existe un estándar federal para los niveles ocupacionales de endotoxinas y β-glucanos en ambientes de interiores. No obstante, varias organizaciones han publicado guías y planes de acción para la calidad del ambiente y aire en interiores. Por lo tanto, el propósito de este estudio fue caracterizar el posible riesgo de exposición a endotoxinas y β-glucanos en edificios ocupacionales que tuvieron daños por agua como resultado del huracán María en Puerto Rico. Durante el mes de junio de 2019, se recolectaron muestras de polvo en el interior de 13 áreas de edificios universitarios con diversos grados de daños por agua y ubicados en cuatro localidades distintas. Las muestras de polvo en superficies horizontales (escritorios) y verticales (paredes) se obtuvieron con hisopos de poliuretano estériles. Las concentraciones de endotoxinas y β-glucanos presentes en las muestras de polvo se determinaron utilizando los ensayos cinéticos de Lisado de Amebocitos de Limulus y Glucatell,

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respectivamente. Finalmente, para determinar el riesgo de exposición, evaluamos las concentraciones de endotoxinas y β -glucanos presentes en las áreas estudiadas y las comparamos con otros estudios para determinar si estos componentes microbianos representan un riesgo para la salud de los trabajadores.

Palabras claves: Exposición ambiental, evaluación ocupacional, daños por agua, calidad ambiental interior, componentes microbianos.

Introduction

Natural disasters are catastrophic events that elicit health, social and economic consequences (Watson, Gayer, & Connolly, 2007). These events include extreme precipitation, floods, droughts, storms, hurricanes and extreme temperature among others. Hurricanes are low-pressure weather systems with sustained winds of > 74 miles per hour (mph) and excessive rainfall. For this reason, hurricanes can severely impact infrastructure due to high winds and floods that result in water damage. During the last 15 years, the United States and Puerto Rico have been impacted by high-intensity hurricanes causing devastating problems to the economy, public health, ecosystems and infrastructure.

In August and September 2005, hurricanes Katrina and Rita, respectively impacted the Gulf Coast of Louisiana, which flooded about 80% of the city of New Orleans lasting for several weeks (Rao et al., 2007). According to Pistrika & Jonlman (2010) more than 200,000 homes became uninhabitable, damaged or destroyed by these hurricanes. In addition, the Centers for Disease Control and Prevention (CDC), estimated that more than 100,000 homes had significant mold growth (Rao et al., 2007). In 2017, another high-intensity hurricane that impacted the US was Harvey, which made landfall in Texas as a category 4 hurricane with winds of 130 mph. Hurricane Harvey unleashed more than 33 trillion gallons of water of rain causing a massive flood event that surpassed the economic damage caused by Katrina (Shultz & Galea, 2017). In the same year, Puerto Rico was impacted by two hurricanes, Irma and María, within a two-week period. The National Oceanic and Atmospheric Administration (NOAA) classified these hurricanes as category 5 and 4, respectively, based on the sustained wind velocity using the Saffir-Simpson scale (Pasch, Penny, & Berg, 2019). Both hurricanes caused significant direct and indirect damage to electrical structures, residences, roads and other infrastructure. Over 30 municipalities were declared disaster zones after the hurricanes (Rodríguez-Díaz, 2018). María is considered the most destructive hurricane impacting Puerto Rico with an estimated damage in housing and other structures fluctuating between \$13 to 16 billion dollars (Asociación de Industriales Puerto Rico, 2017). It is also the third costliest hurricane in the United States (Pasch, Penny, & Berg, 2019).

Hurricane-related water damage can lead to an increase in growth of bacteria and mold in homes and buildings (Chew et al., 2006; Rao et al., 2007). These damp environments can attract others organism such as cockroaches, rodent and dust mites. In addition, the buildings materials that had been damaged with water can release volatile organic compounds (VOCs) (NIOSH, 2012). Microbial growth in indoor environments have been linked to sick buildings syndrome, which is defined by United States Environmental Protection Agency (USEPA) as acute health effects and/or discomfort associated with the time individuals spend in a building where no specific illness or cause can be identified (USEPA, 1991). Several studies have found associations between exposure to bioaerosols and respiratory illness in buildings with water damage (Park et al., 2006; Terr, 2009; Garcia et al., 2013).

Microbial cell components

Many microbial components are classified as Pathogen-Associated Molecular Patterns (PAMPs), which are small molecular motifs expressed endogenously by different classes of microbes and recognized by pattern-recognition receptors (PRRs) present on white blood cells (Thorn, 2001; Beutler, 2004; Mogensen, 2009). These PAMPs can persist in the environment even after remediation processes that effectively kill microorganisms. Among these PAMPs are endotoxins derived from gram-negative bacteria, β -glucans from fungi, lipoteichoic acid of gram-positive bacteria, envelope glycoproteins from viruses and GPI-mucin from protozoa (Mogensen, 2009).

Endotoxins are lipopolysaccharides (LPS) found on the outer membrane of gram-negative bacteria. LPS is ubiquitous in the environment and heat stable (Macher, 1999; Mendy et al., 2018). The LPS involve three major components: O-specific chain, a core oligosaccharide and Lipid A (Figure 1a) (Freudenberg & Galanos, 1993). Endotoxins are recognized by multiple PRRs, including the Toll-Like Receptor 4 (TLR4) which are present on macrophages and dendritic cells, CD14 and lipopolysaccharide binding protein (LBP) which are present on monocytes (Beutler, 2004; Dixon & Darveau, 2005). LPS can promote the release of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-8 (Beutler, 2004; Heine, Rietschel & Ulmer, 2001). Previous studies have found strong associations between endotoxin exposure and respiratory symptoms. In particular, dose-response relationship between endotoxin and acute air obstruction have been observed in humans exposed to cotton dust, with a reported threshold of 90 to 330 Endotoxin Units per cubic meter (EU/m3) of air (Rylander et al., 1985; Castellan et al., 1987; Macher, 1999). Another study reported that the inhalation of 30 to 40 µg of LPS can cause clinical symptoms and changes in pulmonary function (Thorn, 2001). A recent study indicates that doses exceeding 15 to 50 µg of LPS are enough to cause

an inflammatory response and that a dose as low as 2 μ g of LPS was able to cause airway inflammation in non-smoking individuals (Janssen et al., 2013).

 β -glucans are heterogeneous glucose polymers found in fungi cell walls. These fungal polymers contain a backbone of β -(1,3)-linked β -D-glucopyranosyl units with a β -(1,6)-linked side chains (Figure 1b) that vary in distribution and structure length (Akramiene et al., 2007; Noss, et al., 2010). The β -(1,3)-glucans are recognized by different receptors of the immune system such as dectin-1 and Toll-like receptor 2 (Gantner et al., 2003; Noss et al., 2010). Similar to endotoxin, β-glucans can also induce pro-inflammatory responses and the production of cytokines (Douwes, 2005; Noss et al., 2012). In regard of β-glucans exposure multiple studies have shown that inhalation of this fungal component results in respiratory symptoms. Studies conducted in Sweden and Switzerland found that in buildings with visible fungal growth, $(1\rightarrow 3)$ - β -D-glucans concentration ranged from 10 to 100 ng/m3 in airborne dust (Rylander, 1999; WHO, 2009). In 2012, a study determined that concentrations between 25 to 125 µg of glucans can induce the production of pro-inflammatory mediators such as IL-6, IL-1b, IL-8 and TNF-a (Noss et al., 2012). Another study reported that inhalation of 125 ng induced the following symptoms in healthy individuals: throat and nose irritation, sore throat, and headache after 72 hours from the exposure (Thorn, Beijer & Rylander, 2001).



Figure 1. Structural composition cell wall of bacterial endotoxin (A) and fungal β -glucans (B) (Millipore Sigma, 2020; Finkelman, 1998). Regulations of indoor microbial exposure

Currently, there is no federal standard for occupational levels of endotoxin and β -glucans in indoor environments in the US. (Rao et al., 2007; OSHA, 2003). However, the American Conference of Governmental Industrial Hygienists (ACGIH) proposed a relative limit value (RLV) action level of 10 times the

background level for endotoxin when respiratory symptoms are present and a maximum RLV of 30 times above background. ACGIH also emphasizes that active fungal growth in indoor environments is inappropriate (Macher et al., 1999). Moreover, the Occupational Safety and Health Administration (OSHA) suggest that mold should not be present in indoors environments and has published a guide for airborne mold and mold spores in workplaces that includes prevention and remediation plans. In addition, the National Institute for Occupational Safety and Health (NIOSH) and the USEPA have published guidelines and action plans for indoor environmental and air quality (USEPA, 1998; NASEM, 2004; NIOSH, 2012; OSHA, 2003; NIOSH, 2018).

In the Netherlands, the Dutch Expert Committee on Occupational Standards proposed an initial health-based occupational exposure limit for airborne endotoxin of 50 EU/m³ in an eight-hour time weighted average (Heederik & Douwes, 1997), which was reviewed in 2010 to 90 EU/m³ (Health Council of the Netherlands, 2010). Meanwhile, the Institute of Occupational Medicine and Environmental Health (IOMEH) in Poland proposed an occupational exposure limit for endotoxins in industrial indoor air of 200 ng/m³ (2,000 EU/m³) (Brandys & Brandys, 2003). In addition, the Commission of European Communities (CEC) established in 1993 a standard based on research from Sweden, Finland, and the Netherlands. The standard for culturable mold spores in non-industrial indoor workplace including commercial, offices, retail spaces, among others, classify more than 2,000 CFU/m³ as very high, less than 2,000 CFU/m³ as high, less than 500 CFU/m³ as intermediate, less than 100 CFU/m³ as low, and less than 25 CFU/m³ as very low concentrations (Brandys & Brandys, 2003)

Indoor exposure to microorganisms and its components can cause several health problems. Common symptoms or illnesses associated to damp buildings are respiratory symptoms, exacerbation of asthma, hypersensitivity pneumonitis, respiratory infections, allergic rhinitis (hay fever), bronchitis and eczema (NIOSH, 2012). After hurricane María, the Puerto Rican population became more likely to be exposed to microorganisms (i.e. bacteria and mold) and their components in indoor environments. In addition, the slow recovery process forced individuals to live and work in unsafety environments. Therefore, the goal of this study is to evaluate the presence of endotoxins and β -glucans in occupational buildings that had hurricane-related water damage to determine the potential risk of exposure to concentrations known to adversely affect respiratory health.

Materials and methods

In this study, surface dust samples from occupational buildings in a university

setting were obtained and analyzed for the presence of endotoxins and β -glucans, and the potential risk of exposure in buildings that sustained water damage during hurricane María was evaluated.

Sampling sites

Indoor dust samples were collected from buildings located in four different locations of the Universidad Ana G. Méndez. Figure 2 shows in red the municipalities in which the sites are located, two in the north side of the island (i.e. Carolina and Barceloneta) and two in the south (i.e. Santa Isabel and Cabo Rojo). In each site, indoor dust samples were obtained from three areas (i.e. office, library, classroom). Selection of sampling areas at each location was based on the extent of water damage sustained during Hurricane María as reported in the insurance claim by the university. The sustained damage was categorized as none, mild and moderate-to-heavy.



Figure 2. Sampling sites. The municipalities in which the sampled sites are located are shown in red.

Visual inspection and environmental conditions

Indoor dust samples from horizontal and vertical surfaces were collected in June 2019, after Hurricane María made landfall in Puerto Rico. Visual inspection of the areas was conducted during sampling with the aid of the NIOSH Dampness and Mold Assessment Tool to identify potential sources/indicators of damage such as stains, visible mold and wet or damp source in the components of the room or area to be sampled (NIOSH, 2018). During the sampling process, the EVM-7 Environmental Monitor (3M EVM Series, Oconomowoc, WI) was used to measure temperature (°F), relative humidity (%), and CO2 levels (ppm) in each area.

Surface sampling

Indoor dust on horizontal surfaces (i.e. desks and table) was collected with sterile individually wrapped polyurethane swabs (Swab-its[®], Super Brush LLC, Springfield, MA). At each area, dust samples (surface area = 100 cm²) were obtained in triplicate. For vertical surfaces (e.g. walls), large round foam tip polyurethane swabs (Puritan[®], Puritan Medical Products Company LLC, Guilford, ME) were used to collect the samples. A total surface area of 900 cm² from vertical surfaces were sampled in triplicate. Surface dimensions were recorded on site. Immediately after the sample was taken, the swabs were placed in a nonpyrogenic microtube and stored in a plastic bag, transported to the laboratory at room temperature and stored at -20°C upon arrival.

Sample extraction

Dust was extracted from swabs with certified endotoxin-free water. Specifically, samples from horizontal surfaces were extracted with 1 mL, and for vertical surfaces with 3 mL. Samples were sonicated for 30 min and centrifuged at 1400 rpm for 10 minutes (Thorne, 2000). Subsequently, dust extracts supernatants were aliquoted and stored at 4°C for future analysis.

Limulus amebocyte lysate (LAL) assay

Endotoxin quantification was performed with the LAL assay (Pyrochrome, Associates of Cape Cod; Falmouth, MA) according to the manufacturer's instructions. In this assay, a four-fold serial dilution of a control standard endotoxin (CSE) was used to prepare a seven-point calibration curve ranging from 50 to 0.01 endotoxin units per mL (EU/mL). The endotoxin-free water was used as negative control, laboratory and field blanks swab extracts were also used as controls. Each sample was assayed in duplicate. Absorbance was measured at 405nm in intervals of 1 minute for 90 minutes at 37°C using a Synergy[™] HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT). The optical density (OD) threshold to reach an onset time was set at 0.06. Endotoxin concentrations were calculated from a linear regression of the log onset time versus the log concentration of CSE.

(1,3)-Beta-D-Glucan detection assay

The Glucatell assay was used to measure the concentration of β -glucans present on dust samples. The (1,3)- β -D-Glucan standard curve ranged between 3.125 pg/mL and 100 pg/mL. The endotoxin-free water was used as negative control, laboratory and field blanks swab extracts were also used as controls. Each sample was assayed in duplicate. The absorbance was measured at 405nm in intervals of 1 minute for 90 minutes at 37°C using a SynergyTM HTX Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT). The optical density (OD) threshold to reach an onset time was set at 0.06. β -glucans concentrations were calculated from a linear regression of the log onset time versus the log concentration of the (1,3)- β -D-Glucan standards.

Data analysis

Descriptive statistics were used to analyze the distribution of endotoxin and β -glucans in dust samples. To compare the concentrations among samples from different buildings and procedures, a one-way analysis of variance (ANOVA) was used followed by the post-hoc Tukey test for multiple comparisons. All analyses were conducted with the statistical software GraphPad Prism 6.0.

Systematic review

A systematic review was performed according to the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) (Moher et al., 2015) as part of the risk assessment for endotoxin and β -glucan exposure. The PubMed Central (US National Institutes of Health/National Library of Medicine) and Science Direct (Elsevier) databases were used to search relevant literature published between the years 2005 and 2020. The key terms used for the literature search were endotoxin and β -glucans in combination with indoor exposure, occupational, water damage, and respiratory symptoms. Inclusion criteria were occupational workplaces and schools. The articles types included were primary article, review, and case report. The exclusion criteria were household environments and agricultural/industrial occupational settings such as a textile, sewing, poultry and wood. All articles were transferred to the reference management software Mendeley (Elsevier) and duplicates studies were removed. Articles that complied with the inclusion criteria were selected for data extraction and summarized by author, publication year, sampling technique, endotoxin and β-glucans concentrations, and reported respiratory symptoms.

Future directions

Data obtained from this study will be used to conduct an environmental risk assessment of occupational indoor exposure for endotoxin and β -glucans to determine if the concentrations at which these microbial components are found represents a health risk for the workers. Based on the risk assessment findings, recommendations will be provided to remediate the affected areas. Among the recommendations that can be included are periodical visual inspections to identify areas with visible water damage, stain, wet or damp on component in the room such as ceiling, walls, floor, windows, furniture and supplies (books, boxes or equipment). Depending on the findings the recommendations may also include the removal of porous materials such as ceiling tiles, insulation material and wallboards (dry

walls), as well as installing proper engineering controls to monitor environmental conditions (temperature and relative humidity) and maintain proper ventilation levels. The risk assessment and recommendations will be presented to the employer for the implementation of a prevention and/or corrective action plan.

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