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Identifying aerosolized cyanobacteria in the human respiratory tract: A proposed mechanism for cyanotoxin-associated diseases

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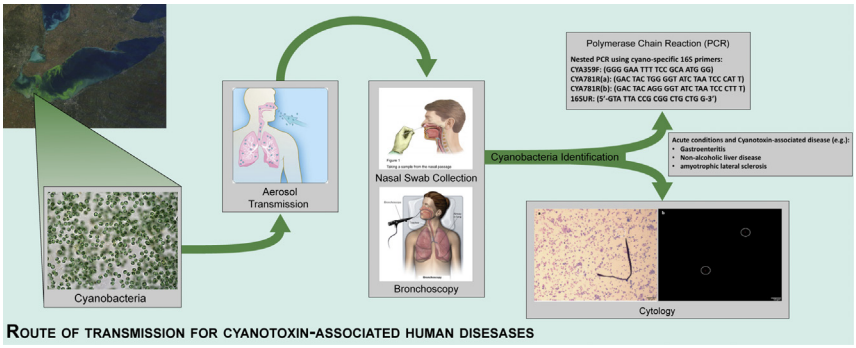
HIGHLIGHTS

- Humans routinely inhale aerosolized cyanobacteria, into the nostrils and lungs.
- Samples collected during the winter were positive at surprisingly high frequencies.
- Proximity to a waterbody was not a significant factor.
- Sources of indoor exposure warrant future investigation.
- Aerosol is a likely route of transmission for cyanotoxin-associated human diseases.

GRAPHICAL ABSTRACT

Route of transmission for cyanotoxin-associated human diseases.



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ABSTRACT

Cyanobacteria produce harmful toxins that have been associated with several acute conditions and chronic human diseases, like gastroenteritis, non-alcoholic liver disease, and amyotrophic lateral sclerosis. Aerosol from waterbodies appears to be a likely mechanism for exposure. We conducted a study of human biospecimens focused on the cyanobacterial aerosilization process by evaluating the extent to which cyanobacteria can invade the human respiratory tract. Our study suggests that humans routinely inhale aerosolized cyanobacteria, which can be harbored in the nostrils and the lungs. Using PCR, cyanobacteria were found at high frequencies in the upper respiratory tract (92.20%) and central airway (79.31%) of our study subjects. Nasal swabs were not predictive of bronchoalveolar lavage (BAL) when detecting inhaled cyanobacteria. Interestingly, we found no evidence that time of year was a significant factor for cyanobacteria positivity (BAL cytology $p = 1.0$ and PCR $p = 1.0$); (nasal swab cytology $p = 0.051$ and PCR $p = 0.65$). Additionally, we found that proximity to a waterbody was not a significant factor for cyanobacteria positivity in BAL and nasal swabs collected during cyanobacteria bloom season [May–October] ($p = 0.46$ and $p = 0.38$). These data suggest that cyanobacteria exposure may be a prevalent and chronic phenomenon not necessarily restricted to waterbodies alone. Sources of indoor exposure warrant future investigation. Given the widespread prevalence of cyanobacterial exposure in the airway, investigation of the aerosol spread of cyanotoxins, more specifically, is warranted. Our findings are consistent with

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the hypothesis that aerosol is a significant route for cyanobacteria exposure, and thus a likely route of transmission for cyanotoxin-associated human diseases.

1. Introduction

Evidence suggests that humans may be exposed to cyanobacteria, also known as blue-green algae, and their harmful toxins through a variety of mechanisms. Cyanobacteria are ubiquitous organisms that can be present not only in cyanobacterial harmful algal blooms (CHABs), but also all year round in the benthic zone of waterbodies (Berdalet et al., 2015; Berger et al., 2006). Two well-documented routes of exposure include incidental consumption of contaminated water (Carmichael and Boyer, 2016; el Saadi et al., 1995; Falconer et al., 1983; van Apeldoorn et al., 2007) and direct dermatological contact through recreational activities, such as swimming (Carmichael and Boyer, 2016; Drobac et al., 2013; van Apeldoorn et al., 2007). Other routes include dietary consumption of cyanotoxin-contaminated fish (Cazenave et al., 2005; Cazenave et al., 2006; Magalhaes et al., 2001; van Apeldoorn et al., 2007; Xie et al., 2005) or even through, supposedly beneficial, blue-green algae supplements (Dietrich and Hoeger, 2005; Drobac et al., 2013; Gilroy et al., 2000; Roy-Lachapelle et al., 2017). More recently, attention has shifted towards understanding cyanobacteria aerosilization, which could make widespread exposure possible and thus be one of the most significant mechanisms with respect to human health (Backer et al., 2010; Lewandowska et al., 2017; May et al., 2018).

Acute cyanotoxin poisoning may present in a clinically diverse fashion. In cases of direct skin contact, clinical findings may include rashes, hives, and blisters (Drobac et al., 2013). Allergic reactions such as cough, runny nose, and sore throat may also occur (Drobac et al., 2013). Swallowing toxic cyanobacteria may also cause gastrointestinal (GI) distress in the form of gastroenteritis, nausea, diarrhea, pain, vomiting, in addition to associated symptoms like fever and headache (Drobac et al., 2013; el Saadi et al., 1995). Exposure to large quantities of cyanotoxins may also cause acute liver damage and even death, as was the case in the Brazilian hemodialysis center in 1996 (Azevedo et al., 2002; Carmichael et al., 2001; Hilborn et al., 2005; Jochimsen et al., 1998). Studies have documented other serious health concerns associated with cyanotoxin exposure including a significantly increased risk for hepatocellular carcinoma (Fleming et al., 2002) and non-alcoholic liver disease (Zhang et al., 2015).

Recent evidence also suggests that living within 0.5 miles of a waterbody affected by frequent CHABs appears to be a significant risk factor for amyotrophic lateral sclerosis (ALS) in Northern New England (Caller et al., 2009; Caller et al., 2012; Caller et al., 2013; Stommel et al., 2013; Torbick et al., 2014; Torbick et al., 2018). Chronic exposure to the cyanobacteria-derived toxin, β-methylamino-L-alanine (BMAA), has been implicated as a significant risk factor for developing neurodegenerative disease, like ALS, in genetically predisposed individuals (Al-Chalabi et al., 2014; Andrew et al., 2017; Banack and Cox, 2003; Banack et al., 2010; Banack et al., 2015; Bradley et al., 2013; Dunlop et al., 2013; Field et al., 2013; Michaelson et al., 2017; Murch et al., 2004a; Murch et al., 2004b; Pablo et al., 2009; Riancho et al., 2018).

A noteworthy and particular route of interest is through aerosol exposure. This mechanism could potentially explain how individuals nearby a cyanobacteria source are exposed to acute and chronic toxicities. A recent ecological study examined the chemical and biological composition of particulate lake spray aerosol (LSA) produced using freshwater samples from Lakes Michigan and Erie, both of which have experienced an increase in CHAB intensity and frequency (May et al., 2018). Blue-green algae was found to be present in individual freshwater LSA particles suggesting that cyanobacteria may be aerosolized through freshwater wave breaking (May et al., 2018). In a related study, Lewandowska and colleagues found that respirable bioaerosols collected over the Baltic Sea, as well as hundreds of meters inland, harbored cyanobacteria and other related microalgae species at very high frequencies (Lewandowska et al., 2017). The authors suggest that microorganism-infested bioaerosols may present a ubiquitous exposure risk for human health, and further studies are needed in characterizing the ability of aerosolized microorganism in carrying other potential toxins like heavy metals and pesticides (Lewandowska et al., 2017).

In the Florida "red tides," brevetoxins produced by a similarly toxic marine dinoflagellate, *Karenia brevis*, were detected in aerosol samples as far as 4.2 km from the beach of origin (Kirkpatrick et al., 2010) as well as in nasal swab specimens of exposed individuals (Backer et al., 2003; Backer et al., 2005). Respiratory symptoms and inflammatory responses were common among those exposed (Backer et al., 2003). An additional study by Backer and colleagues recruited children and adults who had gone to two California lakes to engage in recreational activities, such as swimming and water skiing, and attempted to detect the cyanotoxin, Microcystin (MC), in plasma and nasal swab specimens (Backer et al., 2010). They found that although plasma MCs were all below detectable limits (using MC-specific enzyme-linked immunosorbent assay [ELISA]), levels in nasal specimens increased from pre-recreational to post-recreational sampling. This suggests that aerosol inhalation is a potentially significant route of exposure (Backer et al., 2010). The authors encouraged future studies to collect nasal swab specimens in order to assess upper respiratory tract susceptibility to cyanotoxin infiltration.

The purpose of our study was to expand upon the cyanobacteria aerosol mechanism by investigating cyanobacterial presence in biospecimens from both the upper respiratory tract and the central airway. Previous studies have primarily used MC-ELISA to detect exposure to cyanotoxins (Backer et al., 2010; Hilborn et al., 2005); however, in a novel method we aimed to identify the bacteria itself and the extent to which it may invade the human respiratory system. We accomplished this by analyzing not only nasal swab specimens but also bronchoalveolar lavage (BAL) fluid from consenting research participants. Our primary objectives were to: a) identify cyanobacteria in respiratory samples, b) study the efficacy of using nasal swab specimens as surrogates to bronchoscopy when identifying cyanobacteria, and c) determine if residential proximity to a waterbody correlates with cyanobacteria identification. Factors such as sample collection time of year and significant medical contributing factors (pulmonary disease, smoking status, etc.) were studied as secondary measures.

2. Materials and methods

2.1. Patient recruitment

Institutional review board (IRB) approval was obtained in March 2015 (Center for the Protection of Human subjects (CPHS) at Dartmouth, IRB #20843) with yearly approval in 2015 through 2018. Patients were recruited from the fall of 2016 through the late winter of 2018 via a collaborative effort between the Departments of Neurology and Pulmonary and Critical Care Medicine at Dartmouth-Hitchcock Medical Center (DHMC). Subjects were identified on the DHMC pulmonology service who were either, a) set for participation in an already ongoing research study involving bronchoscopy (CPHS at