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Molecular analysis of single room humidifier bacteriology

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ABSTRACT

Portable, single-room humidifiers are commonly used in homes for comfort and health benefits, but also create habitats for microbiology. Currently there is no information on home humidifier microbiology aside from anecdotal evidence of infection with opportunistic pathogens and irritation from endotoxin exposure. To obtain a broader perspective on humidifier microbiology, DNAs were isolated from tap source waters, tank waters, and biofilm samples associated with 26 humidifiers of ultrasonic and boiling modes of operation in the Front Range of Colorado. Humidifiers sampled included units operated by individuals in their homes, display models continuously operated by a retail store, and new humidifiers operated in a controlled laboratory study. The V1V2 region of the rRNA gene was amplified and sequenced to determine the taxonomic composition of humidifier samples. Communities encountered were generally low in richness and diversity and were dominated by *Sphingomonadales*, *Rhizobiales*, and *Burkholderiales* of the Proteobacteria, and MLE1-12, a presumably non-photosynthetic representative of the cyanobacterial phylum. Very few sequences of potential health concern were detected. The bacteriology encountered in source waters sampled here was similar to that encountered in previous studies of municipal drinking waters. Source water bacteriology was found to have the greatest effect on tank water and biofilm bacteriology, an effect confirmed by a controlled study comparing ultrasonic and boiler humidifiers fed with tap vs. treated (deionized, reverse osmosis, 0.2 μm filtered) water over a period of two months.

1. Introduction

Nearly 10 million humidifiers are purchased in the US each year (ConsumerReports.org 2013), primarily to improve household comfort and for health purposes. Modes of operation of household humidifiers include boiler (produces steam via a heating element), cool mist (produces moisture through an impeller or evaporation), and ultrasonic (produces mist through vibration) (USEPA, 1991). Use of humidifiers can reduce static electricity and ease breathing difficulties such as runny noses associated with colds and shortness of breath

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associated with bronchitis (Trenchs Sainz De La Maza et al. 2002). Humidifiers can be used to keep indoor relative humidity in the optimal range of 40–60%, which has been shown to decrease survival of dust mites, viruses, and microorganisms, and to limit off-gassing of building materials (e.g. formaldehyde) (Arundel et al. 1986; Mohan et al. 1998; Trenchs Sainz De La Maza et al. 2002; Myatt et al. 2010).

On the other hand, humidifiers aerosolize their tank waters, potentially along with any attendant particulates and/or microbial constituents present in that water or in biofilms that develop on humidifier tanks, reservoirs, and spouts. Infrequent cleaning, as practiced by the 40% of Americans who rarely or never clean their humidifiers (ConsumerReports.org 2013), has been shown to be associated with increased risk of adverse respiratory symptoms such as "humidifier fever" and "humidifier lung" (Muller-Wening et al. 2006), or extrinsic allergic alveolitis and hypersensitivity pneumonitis (Nakagawa et al. 1995; Yamamoto et al. 2002; Muller-Wening et al. 2006; Lacasse et al. 2012). Specific opportunistic pathogens that have linked humidifiers with respiratory syndromes are the yeast *Debaryomyces hansenii* (Yamamoto et al. 2002), and bacteria including *Elizabethkingia meningosepticum* (*Nakagawa* et al. 1995), *Pseudomonas* spp. (Forsgren et al. 1984; Rylander et al. 1984), *Mycobacteria* spp. in immune-compromised individuals (Lacasse et al. 2012) and *Legionella* spp. (Moran-Gilad et al. 2012). Endotoxins produced by microbes can be present in tap waters and in air treated by humidifiers at concentrations that are capable of inducing respiratory distress (Rylander et al. 1984; Ohnishi et al. 2002; Anderson et al. 2007). Particulates and mineral dusts can also contribute to lung irritation and injury resulting from humidifier exposure (Daftary et al. 2011; Umezawa et al. 2013). Additionally, lung injury and respiratory failure have resulted from inhalation of commercially available chemical humidifier disinfectants (Hong et al. 2014).

Despite the contribution of microbes present in humidifiers to various health conditions, the microbiology of humidifiers and the sources of any such microbiology are not known. Here, Illumina sequencing of the V1V2 region of the SSU rRNA gene and phylogenetic analyses were used to characterize bacterial microorganisms present in single room humidifiers and their feed waters in relation to variables such as mode of operation of humidifier, microenvironment within the humidifier, and source water type. This is the first culture independent (sequence-based) survey of the microbial constituents of home humidifiers.

2. Materials and methods

2.1. Sample collection

Samples for analysis were collected over a two-month period near the end of the high-use winter season within 25 km of the city Boulder, CO. Samples were taken from three usage categories: (1) in-use humidifiers owned by volunteer residents in the Colorado Front Range, (2) in-use humidifiers on display at a retail store, and (3) new humidifiers purchased for controlled experiments. Private and display model humidifiers were sampled once, while humidifiers purchased for the controlled study were sampled every two weeks for two months, starting with the day they were first filled. The new humidifiers were of two modes of operation, two boilers and two ultrasonic. One of each mode of operation was fed with deionized/reverse osmosis/0.2 μm filtered water (DI/RO), and the other was fed with tap water.

For each sampling event, three sample types were collected: (1) water from the tank and reservoir of the humidifier and (2) source water used to fill the humidifier were collected into clean 1 L HDPE Nalgene bottles; (3) swabs of observable biofilm from any component of the humidifier were collected using sterile culture swabs and stored in 1 mL of 1X PBS in 2 mL sterile polypropylene tubes at −80 °C. Water samples were immediately returned to the lab and filtered onto 0.2 μm pore polycarbonate filters to capture microbial constituents, which were stored at −80 °C until further processing. Water collection bottles were soaked for 30 min in sodium percarbonate (B-Brite) dissolved in 0.2 μm filtered DI/RO water, and then rinsed twice with fresh 0.2 μm filtered DI/RO water and allowed to dry prior to sampling. We were unable to collect credible aerosol samples because of dilution of aerosol output with ambient air. Additionally, we did not collect information to discern whether microbes encountered in this study were alive or active. Nonetheless, the function of humidifiers is to aerosolize tank water, so humidifier output is expected to carry portions of the microbiology encountered.

2.2. Cell counts

For cell counts, 45 mL of each water sample was added to 5 mL of 37% formaldehyde and stored at 4 °C. Formaldehyde-fixed cells were filtered onto black 0.2 μm polycarbonate filters, stained with propidium iodide, and counted by direct fluorescence microscopy (Matsunaga et al. 1995). Cell counts were not conducted for biofilm swabs.

2.3. DNA extraction

For purification of genomic DNA from water samples, polycarbonate filters were removed from frozen storage tubes and placed into 2 mL polypropylene DNA extraction tubes containing: 500 μL of 1:1 phenol:chloroform, 500 μL lysis buffer (71.5 mM NaCl, 71.5 mM TRIS pH 8.0, 7.15 mM EDTA, 2.85% SDS dissolved in DEPC-treated sterile water (Fisher)), and 250 μL of a slurry of 0.1 mm silica/zirconium beads (Biospec Products Inc.) in lysis buffer. For purification of bulk genomic DNA from biofilm samples, frozen storage tubes containing swabs stored in PBS were thawed and vortexed vigorously for 1 min. The top 500uL of the aqueous phase was transferred to a DNA extraction tube. Extraction tubes were subjected to mechanical bead-beating for 2 min in a 16-channel bead-beater (Minibeadbeater 16 # 607, Biospec Products) to lyse cells, during which time filters completely dissolved. Tubes were centrifuged for 5 min at 16,000 × *g*. The top 450 μL of the aqueous phase was transferred to a sterile 1.5 mL polypropylene tube. DNA was precipitated by addition of 10 μL 10 mg/mL glycogen, 200 μL 7.5 M ammonium acetate, and 650 μL isopropanol. Tubes were centrifuged for 30 min at 16,000 × *g* to pellet DNA and the supernatant was decanted. Pellets were washed with 1 mL 70% ethanol and centrifuged 5 min at 16,000 × *g*, after