Residential Exposure to Non-tuberculous Mycobacteria: Sources and Associations with Pulmonary Disease

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Abstract

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The human health impacts of non-tuberculous mycobacteria (NTM) in home environments remain poorly understood. To better understand the association between NTM colonization of POU sources in homes and MAC pulmonary disease (MAC PD), we continued a case-control study of Washington and Oregon residents who have been diagnosed with MAC PD and population controls matched by age, gender, and geography. Environmental samples were collected from bathroom faucets, kitchen faucets, shower aerosols, indoor soil, and outdoor soil. Mycobacterial load in these samples was quantified by using bacteriological culture and PCR. NTM isolates obtained from 30 to 40 matched sets of case and control homes (depending on POU source) were quantitatively compared in three conditional logistic regression models with varying levels of control for confounding. An age-adjusted conditional logistic regression analysis of NTM isolates from shower aerosols shows that the homes of cases had higher odds (odds ratio, 3.67, 95% confidence interval, 1.16-11.53) of having NTM colonization compared to the homes of their matched controls. Associations for the other POU sources remain uncertain;

the odds ratios estimated for other POU sources were consistently greater than one, but with wide confidence intervals, limiting the conclusions that can be drawn without further data.

To better understand what house and homeowner characteristics may affect the colonization of NTM in homes in water POU sources, we also conducted an exploratory analysis utilizing the previous case-control study as a cross-sectional study using various logistic and linear regressions. Associations for various predictor variables relating to house or homeowner characteristics to NTM colonization remain uncertain; however, the large effect estimates and wide confidence intervals suggest that more data in a future study would improve precision and potentially be able to better detect associations.

To our knowledge, this is the first etiologic epidemiological study on the association between NTM colonization of a specific household site and human disease. Previous studies were unable to connect the exposure site to pulmonary disease. The results implicate shower aerosols as a potentially significant source of NTM exposure in homes. While we were unable to detect associations between house and homeowner characteristics and NTM colonization in our exploratory analysis, these characteristics should be included in future study designs with the help of a qualified technical expert.

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Chapter 1: Introduction and Aims

A. NTM Epidemiology

Non-tuberculous *Mycobacterium* (NTM) species are opportunistic and environmentally acquired bacterial pathogens. NTM represent a diverse group of nearly 140 species within the *Mycobacterium* genus (González-Pérez et al., 2013). While they do not cause tuberculosis like their more well-known cousin *M. tuberculosis*, infection of susceptible hosts by NTM species can result in a chronically debilitating pulmonary disease with some similarities tuberculosis, called NTM pulmonary disease (NTM PD). Additional manifestations can include lymphadenitis, soft tissue or skin lesions, and disseminated disease (González-Pérez et al., 2013).

Risk factors for developing NTM PD include advanced age, skeletal anomalies, pre-existing pulmonary diseases, and autoimmune diseases (Mirsaeidi, Farshidpour, Allen, Ebrahimi, & Falkinham, 2014; Dirac et al 2012). There is a greater prevalence in specific populations including females and the elderly (Prevots et al., 2010), or more specifically, non-smoking elderly females with slender body types (Mirsaeidi et al., 2014).

Rising prevalence of NTM PD has been asserted by various authors studying populations in Europe, North America, Central and South America, and Africa (Prevots & Marras, 2015). One of the most common NTM groups found in cases of pulmonary disease in the United States is *M. avium complex (MAC)*, a cluster of closely related species including the human pathogens *M. avium* and *M. intracellulare*. MAC species are increasingly being detected in the environment and may give concern for future health outcomes (Griffith et al., 2007; Honda et al., 2016; Nishiuchi, Iwamoto, & Maruyama, 2017). The true prevalence or incidence of the disease can

be difficult to elucidate, in part due to the gradual onset of symptoms, the overlapping with symptoms of other pulmonary diseases, and lack of reporting (González-Pérez et al., 2013; Mirsaeidi, Farshidpour, Allen, Ebrahimi, & Falkinham, 2014). Despite this challenge, many authors have asserted in the literature that there is increased prevalence of NTM PD (Adjemian et al., 2012, Prevots and Marras, 2015). Increase in measured prevalence may be due to the increasing elderly population in addition to the better detection methods.

In the United States, few studies of NTM epidemiology have tested potential associations between residential exposure to NTM and/or MAC. Several studies have found NTM in water distribution systems and pipes in residential buildings (Falkinham et al., 2015; Falkinham et al., 2008; Nishiuchi et al., 2007, Ristola et al., 2015, Falkinham et al., 2015; Feazel et al., 2009; Thomson et al., 2013; Nishiuchi et al., 2017). Many of these studies have focused on case-only samples of the population. For example, the Nishiuchi 2007 study looked only at the bathrooms of pulmonary MAC patients. NTM were found, however the lack of a control group prevented the study from connecting this exposure to disease risk. The authors could not determine whether the mycobacteria found in the home represented unusual exposures that might be dangerous to susceptible human hosts.

B. NTM in the Environment

Mycobacteria are normal inhabitants of the environment. Many species of NTM have been detected in or near water sources, including soils, lakes, rivers, swamps, and streams (Falkinham, 2015). Environmental mycobacteria exhibit great hardiness against environmental challenges. Pathogenic and non-pathogenic NTM grow, persist, and re-colonize in premise plumbing, defined as the water distribution system and pipes downstream of water meters in

residential buildings and workplaces. NTM resist biocide treatments (Falkinham et al., 2015), UV irradiation (Shin et al., 2008), ozonation and filtration (Hillborn et al., 2006). For example, NTM have been found to be 40-fold more resistant to chlorine than *Pseudomonas aeruginosa* and 100-fold more resistant than Escherichia coli. Their hardiness is also due in part to their mycolic acid-containing and lipid-rich cell envelope. Mycobacteria can be more resistant than other bacteria to chlorine and other biocides due to their unique cell envelopes and their propensity to coagulate in slimy formations and adhere to plumbing surfaces (Falkinham, 2015; Primm et al., 2004; Freeman et al., 2006). Their ability to form biofilms with other microorganisms (Feazel et al., 2009; Mullis and Falkinham 2013) may allow them to persist in diverse environments after leaving water treatment facilities (Dailloux et al., 1999).

As a result of this biofilm formation and hardiness, while many bacteria are well controlled by drinking water treatment, mycobacteria can persist and recolonize in water distribution systems (Falkinham, 2015). NTM are commonly found in treated waters, such as recirculating hospital water sources, biocide-treated metalworking fluids, and showers in residential homes (Primm et al., 2004). For example, one study collected 528 water samples from eight water distribution systems, including their source and effluent, and 135 water samples yielded isolates identified as *Mycobacterium* spp. *M. avium* was found downstream of the water treatment plant and *M. intracellulare* was found in the nearby biofilms despite the use of multiple disinfectants or biocides in drinking water treatment plants (Falkinham, 2001).

Mycobacteria interact with other organisms to increase survival, particularly through the formation of biofilms. Biofilms are thought to contribute to the persistence and presence of NTM in human built infrastructure. For example, a study conducted at a hospital therapy pool found the majority of bacteria present in biofilms near the pools (Angenent, Kelley, St Amand, Pace, &

Hernandez, 2005). Biofilms usually consist of a surface accumulation of strata of microbes which help each other in a complex matrix and ecosystem. Some studies have shown that mycobacteria are important in the early formation of the biofilms and attract other bacteria to associate in the early development of the basal strata (Primm et al., 2004). Biofilms have been shown to increase resistance to chemical disinfection and microbial grazing. Biofilms containing mycobacteria have been found in water distribution pipes to and from drinking water treatment plants and household piping (Falkinham, 2015).

C. NTM in Premise Plumbing and other Point-of-Use (POU) Sources in the Home

Due to the diversity of environmental niches of NTM, there are multiple possible routes for human exposure in the home. Specific household water fixtures have been hypothesized to play important roles in NTM exposure. In particular, *Mycobacterium* species that may partition into water aerosols could conceivably deliver effective infectious doses (Feazel et al., 2009; Primm et al., 2004). Specifically, one study conducted at a hospital therapy pool found NTM in the air above the pool and validated that mycobacteria may be preferentially able to partition into aerosol as the mechanism for disease transfer (Angenent et al., 2005). Studies have focused on sampling for NTM in showerheads, shower water, bathtub water, drain outlets (Falkinham et al., 2015; Falkinham et al., 2008; Nishiuchi et al., 2007), and shower aerosols (Falkinham et al., 2015; Feazel et al., 2009; Thomson et al., 2013; Nishiuchi et al., 2017), other residential hot water sources (Ristola et al., 2015), and faucets (Slosarek et al., 1993, Thomson et al., 2013). Additionally, studies of MAC PD patients have found MAC isolates in potting soil (De Groote et al., 2006).

Showers or baths may be an important route of transmission for non-tuberculous mycobacteria because of the potential for repeated exposure to aerosolized microbes in enclosed spaces. The showerhead is a niche that provides a dark, wet, warm, environment that is frequently replenished with low-level nutrient resources and organisms. This makes the showerhead an environment that is favorable for microbial growth, including MAC and other NTM. One study has hypothesized that the rise of pulmonary infections by NTM may be in part due to the increased use of showers rather than baths (O'Brien et al., 2000). Another study found Mycobacteria in biofilms of the showerheads at more than 100-fold above background water samples (Feazel et al., 2009). Based on this evidence, one expert recommends changing the showerhead once per year or more frequently in order to decrease exposure (Mann, 2009); however, few do, which may be another reason why showers are an important route of transmission.

D. Relevant previous work by Cangelosi Lab

The Cangelosi lab has studied and published on non-tuberculous mycobacteria and environmental and host risk factors using a population based case-control study. The study was conducted in two phases: a first interview phase and a second environmental sampling phase. The first phase, previously published, interviewed subjects about host behaviors (including interactions with water and soil), host susceptibility factors, medical history, and home features. At the time of the interview, environmental samples were collected for the second phase, which forms the first aim of this dissertation. Phase one of the study found that that one behavior, spraying plants with spray bottles, was associated with MAC PD (MAC pulmonary disease) (Dirac et al., 2012). Host susceptibility factors were also assessed including medical history,

BMI, and other comorbidities. Several of these risk factors exhibited strong associations with MAC PD in the study. These included body mass index (BMI), thoracic skeletal abnormality, pneumonia, chronic obstructive pulmonary disease (COPD), steroid usage, and immunomodulatory drug usage (Dirac et al., 2012). Host home features looked at the age of the home, well usage, air-conditioning usage, and use of in-line or pitcher filters. If there was an association with these factors, it may have been too subtle for the study to detect. The study was able to detect those associations with a greater effect size and was underpowered for more subtle effects. Overall, the study indicated that host susceptibility factors were more important to MAC PD disease risk than environmental factors. However, the importance of environmental factors was not excluded, and this is further explored in phase two.

Accordingly, in Aim 1 of this dissertation, we continued the environmental sampling phase of the case-control study and analyzed whether NTM PD is associated with colonization of NTM in faucets, showers, and soils. For Aim 2, we asked a different, yet related question in a secondary analysis and explored how home features, including decade of house construction, remodeling, frequency of showering or bathing activity, geography, and demographic variables, are associated with colonization of faucets and shower aerosols with NTM.

E. Innovation

This dissertation enhanced our understanding of the health consequences of environmental exposure to MAC and other NTMs. Aim 1 of the dissertation was the first and most inclusive to measure MAC and NTM occurrence in the home environment and associate this with human health. To our knowledge, this was the first case-control study of NTM pulmonary disease conducted on a general (non-hospital based) population. The current

understanding for MAC PD is based on host-related risk factors. Results of Aim 1 of this dissertation has the potential to create more targeted strategies for protecting vulnerable populations. The conclusions may also be communicated to various disciplines related to the built environment, such as building scientists, architects, and who can make informed decisions about building and maintaining healthy homes. Additionally, these conclusions may be communicated to community health workers, housing authority, and other community officials who influence policies and help the community maintain healthy homes.

F. Specific Aims

Given the increasing prevalence of MAC pulmonary disease and other NTM infections in the United States, there is a need to more fully understand the connection between residential exposure and disease, as well as the environmental factors in residences that can lead to environmental exposure in patients. This dissertation addresses these two aspects, first using a case-control study in a generalizable population that assessed multiple point-of-use (POU) sources of infection in the home environment to assess the association between exposure and disease. In the second part, we conducted an exploratory analysis to identify specific house characteristics, which may affect NTM colonization in residential environments.

To this end, we used samples taken from the homes of clinically diagnosed MAC PD patients in Washington and Oregon, and from the homes of matched controls, to investigate the relationship between domestic environmental exposure to NTM and MAC pulmonary disease status. Our exploratory analysis asked how certain house characteristics affect the growth of NTM in POU water sources in the home environment. The overall objective is to determine whether the home environment has a role in NTM and MAC disease in a sample of the population outside of healthcare settings. Our specific aims are the following:

Aim 1: Determine whether there is an association between MAC colonization of homes and disease status. This was accomplished through a case-control analysis comparing the presence or absence of the bacteria in multiple samples from the homes to disease status of occupants. *We hypothesized that the homes of those who are clinically diagnosed with MAC PD have greater detectable MAC and other NTM colonization than the homes of age, gender, and geographically matched controls.*

Aim 2: Explore whether house and homeowner factors affect the mycobacterial colonization of homes. This was a cross-sectional analysis. *We hypothesized that variables including the decade of construction, aspects of the premise plumbing, geography, and demographic factors of the homeowners are associated with NTM colonization in water POU sources in the home.*

Chapter 2: Aim 1

A. Abstract

Background: Non-tuberculous mycobacteria (NTM), including *Mycobacterium avium* complex (MAC), can opportunistically cause debilitating pulmonary disease in susceptible human hosts. Point-of-use (POU) water sources in homes are potential sources of acquisition. The human health impacts of NTM in home environments remain poorly understood.

Objectives: This study tested the association between NTM colonization of POU water sources in homes and MAC pulmonary disease (MAC PD).

Methods: A case-control study was conducted of Washington and Oregon residents who have been diagnosed with MAC PD, and population controls matched by age, gender, and geography. Environmental samples were collected from bathroom faucets, kitchen faucets, shower aerosols, indoor soil, and outdoor soil. Mycobacterial load in these samples was quantified by using bacteriological culture combined with PCR. Numbers of NTM isolates obtained from 30 to 40 matched sets of case and control homes (depending on POU source) were quantitatively compared in three conditional logistic regression models with varying levels of control for confounding: a crude/unadjusted model; an age-adjusted model (with age as a continuous variable); and a race/ethnicity, education level, and age-adjusted model. Our primary analysis of interest is the age-adjusted model.

Results: Larger numbers of NTM were isolated from shower aerosols collected in case homes than in control homes. An age-adjusted conditional logistic regression analysis of the binary exposure of NTM isolates from shower aerosols shows that the homes of cases had higher odds (odds ratio, 3.67, 95% confidence interval, 1.16-11.53) of having NTM colonization compared to

the homes of their matched controls. Associations for the other POU sources remain uncertain; the odds ratios estimated for other POU sources were consistently greater than one, but with wide confidence intervals, limiting the conclusions that can be drawn without further data.

Discussion: To our knowledge, this is the first etiologic epidemiological study on the association between NTM colonization of a specific household site and human disease. Previous studies suggested shower aerosols as source of interest, but were unable to connect the exposure site to pulmonary disease. The results implicate shower aerosols as a potentially significant source of NTM exposure in homes.

B. Introduction

Certain non-tuberculous mycobacteria (NTM) species are opportunistic and environmentally acquired bacterial pathogens. Infection of susceptible hosts by NTM can result in chronically debilitating pulmonary disease. NTM pulmonary disease (NTM PD) has known host-risk factors which include advanced age (Kendall and Winthrop 2013; Winthrop et al., 2010), thoracic structural anomalies (Dirac et al., 2012; Iseman et al., 1991; Kim et al., 2008; Kartalija et al., 2013; Griffith et al., 2007), pre-existing pulmonary diseases (Dirac et al., 2012; Griffith et al., 2007; Mirsaeidi et al., 2014), and autoimmune diseases (Dirac et al., 2012; Griffith et al., 2007; Mirsaeidi et al., 2014; Chan and Iseman 2013).

Rising prevalence of NTM PD has been reported in North America (Prevots and Marras 2015) since 2000. In the United States, the various studies looking at patients in integrated health systems, Medicare beneficiaries, and the general population report the prevalence ranging from 4.1 and 14.1 per 100,000 person-years (Kendall and Winthrop 2013, Marras et al., 2007, Winthrop et al., 2010, Winthrop et al., 2013, Prevots et al., 2010, Adjemian et al., 2012). Of the NTM, one of the most common causes of pulmonary disease in the United States is *Mycobacterium avium* complex (MAC) However, they do not address residential environments, where many people may acquire the disease.

Opportunistic NTM grow and persist in premise plumbing, defined as the water distribution system and pipes downstream of water meters in residential buildings and workplaces. NTM resist biocide treatments (Falkinham et al., 2015), UV irradiation (Shin et al., 2008), ozonation and filtration (Hillborn et al., 2006). They form biofilms with other microorganisms (Feazel et al., 2009; Mullis and Falkinham 2013) allowing them to persist in

diverse environments after leaving water treatment facilities (Dailloux et al., 1999). Specific household water fixtures have been hypothesized to play important roles in NTM exposure. In particular, *Mycobacterium* species that may partition into water aerosols, and aerosol-generating fixtures such as showerheads could conceivably deliver effective infectious doses (Feazel et al., 2009; Angenent et al., 2005).

Studies of the homes of MAC pulmonary disease (MAC PD) patients have found MAC isolates in potting soil (De Groote et al., 2006), showerheads, shower water, bathtub water; and drain outlets (Falkinham et al., 2015; Falkinham et al., 2008; Nishiuchi et al., 2007), bathtub inlets (Nishiuchi et al., 2009), residential hot water sources (Ristola et al., 2015), and shower aerosols (Falkinham et al., 2015; Feazel et al., 2009; Thomson et al., 2013; Nishiuchi et al., 2017). While these studies identified potential sources of infection in homes, they did not test whether these were associated with disease status. To our knowledge, there have been no previous studies that have tested the association of MAC in environmental samples with disease status by sampling in the residential areas of both those with and without disease.

To understand better the connection between residential exposure to NTM and human health, we quantified *Mycobacterium* isolates from home environments of clinically diagnosed MAC PD patients in Washington and Oregon, and from the homes of age-, gender-, and geography-matched population controls. The objective was to determine whether mycobacterial colonization of specific home environments is greater in the homes of MAC PD cases than in matched control homes. MAC isolation from environmental sources is relatively uncommon in comparison to the broader NTM category. Since both bacteria can survive in similar environments, for our study, we included NTM in the analysis as an indicator of environments that are conducive to colonization by diverse mycobacteria including MAC.

C. Methods

Study Design/Subject Recruitment

This study continued a previous population-based matched case-control study (Dirac et al., 2012) involving adult HIV-negative MAC PD patients in Washington and Oregon. Recruitment of MAC PD cases began in January 2009 and was completed by January 31, 2011. Eligible cases were those who met the 2007 American Thoracic Society diagnostic criteria and visited a provider for MAC PD at least once since 2007. Cases who lived in nursing homes or similar institutions, had cystic fibrosis, or had HIV were excluded (Dirac et al., 2012).

Population controls were identified and recruited between May 2010 and July 2011 by using random-digit dialing. Telephone numbers to call to screen for eligible controls were formed using the first 7 digits of each case's 10-digit primary telephone number and completed with 3 random digits. Cases predominantly reported landlines as their primary phone numbers. The area code (first 3 digits), and telephone prefix or exchange code (next 3 digits) correlate roughly with geography, so this provided our geography-matching. Potential controls were then screened to further match with cases through gender and age groups (Dirac et al., 2012). Age groups for control matching ranged from five years younger than the case to five years older for cases younger than 59 years old, to as much at 15 years younger than the case to 15 years older than the cases for older cases. This was due to the difficulty of finding eligible and willing individuals in the oldest age-groups to serve as controls. A detailed list of age-groups is included in the Appendix as Appendix A.

Institutional review boards at University of Washington and Oregon Health & Science University approved this study.

Recruitment and In-home interview

Once study participants were contacted via telephone, they received a home visit that included informed consent, an interview, and collection of multiple environmental samples from five point-of-use (POU) sources around the house or apartment (Dirac et al., 2012). Participants were asked questions about their health, their homes, behaviors hypothesized to generate aerosol exposures, demographic data, date of diagnosis (for cases) and where the subject lived on the index date. (Dirac et al., 2012).

Environmental Sampling

Environmental sampling was done at five POU sources: bulk water from kitchen taps, bulk water from bathroom taps, shower aerosols, aerosolized indoor soil, and aerosolized outdoor soil.

Bulk water samples were collected from bathroom and kitchen taps in sterile 1-liter Nalgene bottles. Both hot and cold water taps were opened until the stream reached the participants' usual hand-washing temperature, then 1 liter of water was collected.

Samples of outside soil and (if available) inside soil were collected by using an autoclaved hand trowel to transfer material into pre-UV-irradiated plastic bags. Composite samples were collected for both indoor and outdoor.

Shower aerosols were collected from the bathroom most often used by the subject for showering or bathing. If participants reported that they exclusively bathed rather than showering, they were asked to adjust the shower to their usual showering temperature and usual pressure. Then, a BioStage® single-stage cascade impactor powered by an attached Quick-Take 30 High

Flow Pump (SKC Inc, Eighty-Four, PA) was placed in the shower chamber outside of the direct path of the showerheads' spray for 10 minutes (flow rate of 30 L/min). Two 10-minute samples were collected, one with the impactor loaded with a Petri dish with Middlebrook 7H10 agar with OADC enrichment, and the other with the impactor loaded with a Petri dish with Middlebrook 7H10 agar enriched with 0.001% malachite green. If participants exclusively bathed in a tub, participants were asked to fill their bathtub until it reached the participant's usual temperature and level. The impactor and pump were held near the running faucet for 2 minutes while the bath was filling, and then held over the surface of the water in the tub for 8 minutes. If the participants' bath had jets, the procedure was slightly modified by holding the impactor and pump for 2 minutes near the running faucet with jets on, and for 6 minutes over the surface of the water in the tub (Dirac et al., 2012).

Laboratory processing

Laboratory processes including NTM isolation and identification are outlined in Figure 2.1. After collection, environmental samples were transported to the University of Washington Environmental and Occupational Health Microbiology Laboratory (EOHML) in a cooler with ice packs. Microbiologists and PCR technicians in the lab were blinded to the case vs. control status of samples after the creation of primary culture plates.

Primary plating of bulk water samples was accomplished by vacuum filtration. 250 mL of the water samples were poured into a sterile polyphenylsulfone magnetic filter funnel (Pall Life Sciences, Ann Arbor, MI) containing 0.45 μm, 47mm filters (EZ-Pak, MilliporeSigma, Burlington MA). Filters were removed and placed on the primary culture plates with Middlebrook 7H10 agar with OADC enrichment. A second 250 mL of the water samples were

treated with 1.25 mL of 1% cetylpyridinimum chloride (CPC) to decontaminate the aliquot for 30 minutes, vacuum filtered and also plated on Middlebrook 7H10 agar with OADC enrichment.

The Petri dish in the BioStage® cascade impactor constituted the primary culture plate for shower aerosol samples.

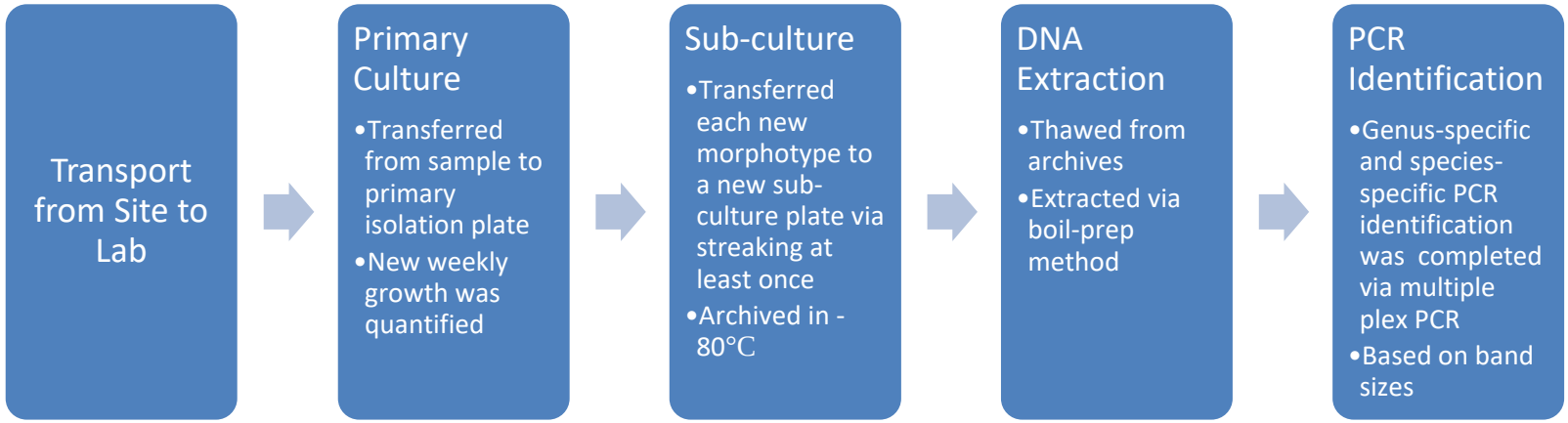

Figure 2.1. Flow diagram of laboratory processes.

For primary plating of soil samples, an autoclavable soil aerosolization chamber (SAC) was designed specifically for this study (Figure 2.2). The chamber was custom fabricated from a 5-gallon milk pail and modified to include a soil delivery cylinder on top and sampling port on the side. A BioStage® single-stage cascade impactor was attached to the side sampling port of the SAC using a stainless steel elbow, gasket and hose clamp. Soil samples from a subject's home were divided into two portions that were separately dropped in through the delivery cylinder. For the first portion the impactor was loaded with a petri plate of Middlebrook 7H10 agar with OADC enrichment and for the second portion with Middlebrook 7H10 agar with OADC enrichment and 0.001% malachite green (wt/vol), modelled after De Groote et al. (2006). The sampling pump was run for 10 minutes after the soil was released at 30 L/min.

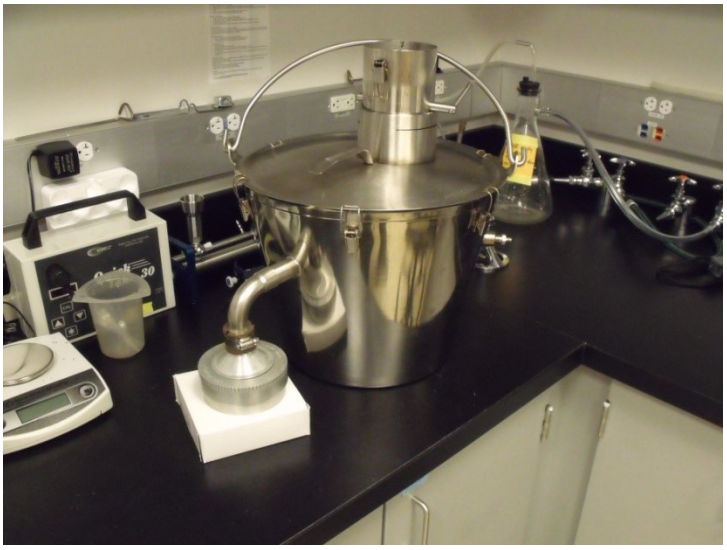

Figure 2.2. Custom-built Soil Aerosolization Chamber (SAC) for generation and collection of soil aerosols prior to primary plating.

Primary plates were incubated in sealed bags at 37 °C and observed for 8 weeks, or until the culture became desiccated or molded. Each week, new colonies and new colony morphotypes were recorded. Each new colony type was transferred by sterile loop to a new sub-culture plate

by streaking for isolation. All sub-culture plates were similarly incubated and observed for 8 weeks or until the culture became desiccated or molded. This process was repeated at least one time, and additional times if the culture grew multiple morphotypes until sub-culture plates only had a single morphotype.

DNA was extracted from purified colonies by a boil-prep, heat-lysis method. Each tube was boiled in a thermocycler at 96 °C for 10 minutes, cooled to 4 °C, and then centrifuged for 2 minutes. Aliquots of each supernatant (5 μL) were then subjected to a multiplex PCR described by Wilton and Cousins (1992). PCR primers from Wilton and Cousins (1992) were used as outlined in Table 2.1. PCR conditions were as follows: 95 °C for 30 seconds followed by 35 cycles of 98 °C for 10 seconds, 62 °C for 15 seconds, and 72 °C for 10 seconds. The resulting PCR products were electrophoresed in 2% agarose gel with 90 volts for 90 minutes. Detected band sizes in the gel were correlated with genus and species identifications as outlined in Table 2.2.

Table 2.1 Expected product size of primer pairs used in the multiplex PCR

Primer pairs	Primer concentrations, respectively (nM)	Product Size (bp)
Mycgen-F and Mycgen-R	250 + 250	1030
Mycgen-F and Mycav-R	250 + 70	180
Mycint-F and Mycgen-R	350 + 250	850
TB1-F and TB1-R	200 + 200	372

Abbreviations: nM, nanomolar; bp, base pair

Table 2.2. PCR product sizes and species interpretation

Species	Detected Product Size (bp)
M. avium	180
M. avium	1030+180
M. avium	180+EB[1]
M. avium	1030+180+EB
M. avium intracellulare	1030+850+180
M. avium intracellulare	850+180+EB
M. avium intracellulare	1030+850+180+EB
M. avium intracellulare	850+180
M. intracellulare	850
M. intracellulare	1030+850
M. intracellulare	850+EB
M. intracellulare	1030+850+EB
Mycobacterium	1030
Mycobacterium	1030+EB
Negative/No species detected	Only EB
Negative/No species detected	No reaction

Abbreviations: bp, base pair; EB, extra bands

Inferential Analyses

Statistical analyses were performed using STATA /SE v. 14.2. Our analysis asked whether there were associations between MAC or NTM colonization and disease status. It was designed to take into consideration case-control matching, and to enable more refined consideration of confounding variables. Associations between disease status and the isolation of bacteria (the variable of interest), were estimated using odds ratios obtained through 3 conditional logistic regression analysis models comparing each case to his/her respective age-, gender- and geography- matched control: an (unadjusted) crude model, an age-adjusted model, and a fully adjusted model. These models have different degrees of control for confounding. The crude model contained only the variables of interest, namely disease status and the isolation of NTM. The age-adjusted model contained the disease status, isolation of NTM, and age as a confounding variable of interest. While age was one of the matching variables utilized during recruitment of cases and controls, we were still concerned about residual bias since age was matched within age-groups of 10 to 30 years. While geography was also one of the matching variables and of potential concern regarding residual bias, we lacked more refined data to be able to address this in our analysis. Gender was more tightly matched in this study, so there was less concern about residual bias. Multiple ways of including age in this model were considered: 1. include age as a continuous variable, 2. include age as a continuous variable but centered on the age categories used when matching cases and controls. A detailed list of age-categories is included in the Appendix as Appendix A. The final model chosen for age in the age-adjusted model was as a continuous variable because there was little difference between the exposure effect estimates and 95% confidence intervals of both the continuous and the centering on categories model.

The fully adjusted model contained the previously mentioned variables of interest from the two models (disease status, isolation of NTM, age as a continuous variable) in addition to other potential confounding variables: race and education level. These 3 models were also applied to two outcomes: MAC isolation as our primary outcome, in addition to the secondary outcome of any NTM isolation. “Isolation of bacteria was analyzed as a binary variable: one or more isolates found versus zero isolates found.

D. Results

The study enrolled a total of 70 cases and 61controls in 52 matched sets (Dirac et al., 2012). Of these, environmental samples were collected from a subset of 56 cases and 51 controls. There were one matched set of four (1 case, 3 controls), four matched sets of three (1 case, 2 controls), and the remainder were matched pairs (1 case, 1 control). Controls were not used more than once. Environmental samples were collected only if the case subject lived in the same home at the time of diagnosis. If either member of a case-control matched set did not have a sample from a specific POU source (e.g. indoor potting soil), that matched set of site-specific samples was not included in the matched analysis, which resulted in different numbers for each POU source. As a result, the total amount of samples from the various POU sources are the following: we analyzed the shower aerosols of 39 cases and 46 controls in 39 matched sets, the bathroom faucets of 40 cases and 48 controls in 40 matched sets, the kitchen faucets of 40 cases and 48 controls in 30 matched sets, the indoor soil of 30 cases and 38 controls in 30 matched sets, and the outdoor soil of 39 cases and 46 controls in 39 matched sets.

The characteristics of the case and control samples are shown in Table 2.3. Numbers in this table include all matched participants with environmental samples, including those that were

not included in the conditional logistic regression analysis for some POU sources. In this table, all variables are categorical except for age and they are presented with the percentage and number of the categories within each characteristic. Since age was analyzed as a continuous variable, it is presented in this table with the median and interquartile range (IQR). Median was selected because age did not follow a normal distribution. To compare between the case and control medians, a Mann-Whitney U test was completed. A chi-square test was used to compare the categorical characteristics of cases and controls and see if any characteristics were different between the two samples.

We found no difference in these characteristics between cases and controls. Demographically, the cases and controls recruited in this study were predominantly elderly, highly educated, non-Hispanic white females. Most cases resided on the western side of the state, following the population distribution of Washington and Oregon. By design, the gender, age, and geography make-up of the controls was similar since cases and controls were matched on those variables.

Table 2.3. Demographic characteristics of MAC cases and controls.

Characteristic	Case count (%), N =40	Controls count (%), N = 48	P-value
Gender			0.92
Female	33 (82.50)	40 (83.33)	
Male	7 (17.50)	8 (16.67)	
Age			0.21
Median (IQR)	69 (16.00)	64.5 (12.50)	
Race/Ethnicity			0.22
White, Non-Hispanic	37 (92.50)	46 (97.87)	
Black, Non-Hispanic	0 (0.00)	1 (2.13)	
Asian or Pacific Islander, Non-Hispanic	2 (5.00)	0 (0.00)	
Native American, Non-Hispanic	1 (2.50)	0 (0.00)	
Hispanic	0 (0.00)	1 (2.13)	
State			1.00
Washington	30 (75.00)	36 (75.00)	
Oregon	10 (25.00)	12 (25.0)	
Side of State			0.85
Western	38 (95.00)	46 (95.83)	
Eastern	2 (5.00)	2 (4.17)	
Education			0.68
High School or equivalent	8 (20.51)	6 (12.77)	
Some college	5 (12.82)	10 (21.28)	
Associates	4 (10.26)	4 (8.51)	
Bachelors	7 (17.95)	12 (25.53)	
Masters	8 (20.51)	10 (21.28)	
Professional or Doctorate	7 (17.95)	5 (10.64)	

We compared the mean numbers of NTM isolates from case and control homes in Table 2.4. As expected given that MAC is a sub-category of NTM, numbers were larger for NTM (Table 2.4) than for MAC isolates (Table 2.5). The broader NTM category was included in the analysis because diverse environmental *Mycobacterium* species are likely to thrive in similar environments to MAC. More NTM were isolated from case homes than from control homes in all POU sources (Table 2.5).

Table 2.4. NTM isolated from POU sources. Numbers reported are means of counts of isolates.

POU source	N (case, control)	Case		Control	
		Mean	SD	Mean	SD
Bathroom faucet	40, 48	5.35	12.17	1.97	0.59
Kitchen faucet	40, 48	3.98	7.60	3.75	6.42
Shower aerosols*	39, 46	2.64	5.84	0.55	1.83
Indoor soil (e.g. potted plants)	30, 38	3.23	6.33	2.43	6.11
Outdoor soil (e.g. yard)	39, 46	1.28	2.65	0.96	2.35

Abbreviations: NTM, nontuberculous mycobacteria; POU, point-of-use
*Shower aerosols were found to be statistically significant via a paired t-test at (p-value, 0.0362)

Table 2.5. MAC isolated from POU sources. Numbers reported are means of counts of isolates.

POU source	N (case, control)	Case		Control	
		Mean	SD	Mean	SD
Bathroom faucet	40, 48	2.03	4.76	0.97	2.58
Kitchen faucet	40, 48	1.60	3.10	1.83	4.01
Shower aerosols	39, 46	0.82	2.65	0.14	0.34
Indoor soil (e.g. potted plants)	30, 38	0.57	1.38	0.97	4.39
Outdoor soil (e.g. yard)	39, 46	0.39	0.91	0.44	1.14

Abbreviations: NTM, nontuberculous mycobacteria; POU, point-of-use

Our study matched cases with controls on the basis of three demographic characteristics, age, gender, and geography (as indicated by the same telephone area code and prefix). To estimate the association between pulmonary disease and NTM colonization in five POU sources, there were 3 models, each producing an effect measure, odds ratios, and 95% confidence intervals (Table 2.5). Our first model, a crude, unadjusted conditional logistic regression, asked whether isolates from any POU source hypothesized to be involved in exposure to NTM was positively associated with disease. The odds of having NTM isolated from their shower aerosols was higher among those with MAC PD than their matched controls (odds ratio, 3.17, 95% confidence interval, 1.13-8.86).

Our second model was an age-adjusted conditional logistic regression analysis. As shown in Table 2.6, the odds of having NTM isolated from their shower aerosols was higher among those with MAC PD than their matched controls (odds ratio, 3.66, 95% confidence interval, 1.16-11.53). Our third model was a fully adjusted conditional logistic regression analysis, which considered not only age, but also race and education as potential confounders. As shown in Table 2.6, the odds of having NTM isolated from their shower aerosols was higher among those with MAC PD than their matched controls (odds ratio, 3.99, 95% confidence interval, 1.19-13.45). For bathroom faucet, kitchen faucet, indoor soil, and outdoor soils, we were unable to detect associations between NTM isolates and disease.

Table 2.6. Summary of conditional logistic regressions for NTM colonization in five POU sources in the home, adjusted for age and potential confounding variables (race and education).

POU source	N (case, control)	OR and 95% CI for crude model		OR and 95%CI for age-adjusted model		OR and 95% CI for fully-adjusted model, adjusted for age, race, and education	
Bathroom faucet	40, 48	1.74	0.75-4.00	1.67	0.67-4.18	2.09	0.80-5.48
Kitchen faucet	40, 48	1.64	0.68-3.95	1.29	0.49-3.38	1.57	0.59-4.17
Showerhead aerosols	39, 46	3.17	1.13-8.86	3.66	1.16-11.53	3.99	1.19-13.45
Indoor soil (e.g. potted plants)	30, 38	1.98	0.72-5.44	1.65	0.57-4.80	1.43	0.46-4.43
Outdoor soil (e.g. yard)	39, 46	1.23	0.44-3.41	1.07	0.36-3.18	1.17	0.40-3.41

Table 2.7. Summary of conditional logistic regressions for MAC colonization in five POU sources in the home, adjusted for age and potential confounding variables (race and education).

POU source	N (case, control)	OR and 95% CI for crude model		OR and 95%CI for age-adjusted model		OR and 95% CI for fully-adjusted model, adjusted for age, race, and education	
Bathroom faucet	40, 48	1.76	0.69-4.53	2.16	0.71-6.55	2.21	0.71-6.91
Kitchen faucet	40, 48	1.16	0.51-2.63	1.16	0.47-2.85	1.28	0.50-3.30
Showerhead aerosols	39, 46	2.65	0.67-10.43	2.81	0.62-12.74	2.90	0.61-13.94
Indoor soil (e.g. potted plants)	30, 38	1.87	0.54-6.43	1.33	0.35-5.08	1.22	0.32-4.72
Outdoor soil (e.g. yard)	39, 46	1.00	0.32-3.10	0.96	0.29-3.17	0.95	0.28-3.18

Shower aerosols were significantly associated with disease whether or not variables were adjusted in the regression model with respect to NTM colonization. Upon comparing all three models together, as shown in table 2.6, we are able to see that for shower aerosols, adjustment for any of the potential confounders did not considerably affect the magnitude of the effect estimates. When looking more specifically at MAC colonization at POU sources, we were unable to detect associations between MAC isolates and disease in all POU sources. (Table 2.7).

E. Discussion

Our study found more NTM colonization in case homes compared to homes of age, gender, and geography-matched controls. We also found that NTM colonization in shower aerosols were significantly associated with MAC pulmonary disease.

To our knowledge, this is the first observational study of associations between human health and exposure to NTMs in the residential environment. Although NTM diseases such as MAC PD are likely to be driven mainly by host risk factors, this study found evidence for environmental exposure factors in homes.

Our findings expand on previous studies that have found NTM to persist in premise plumbing, (Falkinham et al., 2008, Mullis and Falkinham 2013, Dailloux et al., 1999) can be isolated from showerheads and shower aerosols, (Falkinham et al., 2015, Falkinham et al., 2008, Nishiuchi et al., 2007, Feazel et al., 2009, Ristola et al., 2015, Thomson et al., 2013, Nishiuchi et al., 2017) and do so preferentially compared to other organisms (Angenent et al., 2005), as previously described in more detail in Chapter 1. NTMs have previously been isolated from

showerheads and shower aerosols, potentially resulting in airborne exposure (Angenent et al., 2005; Falkinham et al., 2008; Nishiuchi et al., 2007; Ristola et al., 2015; Thomson et al., 2013). An additional study identified shower aerosols (Feazel et al., 2009) as a reservoir in the home for diverse opportunistic infectious agents, including NTM. NTMs have been documented to persist in biofilms (Falkinham et al., 2008; Mullis and Falkinham 2013) or generally in premise plumbing despite controls such as disinfectants or filters (Falkinham et al., 2008; Dailloux et al., 1999). Angenent et al., (2005) found that *Mycobacterium* species preferentially partitioned into aerosols compared to other bacteria species. Conceivably, mycobacteria in premise plumbing can detach from biofilms and aerosolize at showerheads, effectively seeding the airways of users. This hypothesis, previously based solely on the observed presence of NTMs in showerheads and aerosols, is now greatly strengthened by our disease association findings.

We could not detect associations with other POU sources. The effect estimates for NTM colonization of all the POU sources were above 1, suggesting association, but our confidence intervals reveal our data are consistent with a wide range of effects some of which may be substantial. If there were a true association, it may be more subtle than the association found in the shower aerosols, requiring greater power to detect. More data would help improve precision. Better distribution across key covariates will also improve efficiency of the study. As a result, right now, the associations remain unknown. We need future studies on residential exposures in the home with larger numbers to determine whether or not NTM colonization at the other POU sources are in fact associated with disease.

There is no evidence that the species and strains of environmental mycobacteria isolated in this study were involved in initial MAC PD infections. We were unable to genotypically match environmental MAC isolates with clinical isolates since only a small number of clinical

isolates were available to study. Most of the environmental NTM isolates observed here were likely to be species other than MAC. Moreover, the MAC PD cases included in this study were diagnosed with their diseases as early as 1997, and initial infections may have occurred earlier than that, while sampling took place from 2009 through 2011. However, this study did indicate that shower aerosols and associated plumbing were more heavily colonized with NTMs in case homes than in control homes. This may reflect a more favorable environment for colonization by diverse *Mycobacterium* species, a condition that could increase the probability of exposure to specific disease-causing species such as MAC. Alternatively, chronic exposure to NTMs (including but not limited to MAC) could exacerbate an existing MAC PD infection, either through reinfection or by stimulating immune responses leading to symptoms. Chronic NTM exposure and a corresponding escalating immune response could even prime the host for primary MAC infection (e.g. through macrophage recruitment). This would increase the likelihood of diagnosis and inclusion in our case group. An association with MAC could be consistent with cases contaminating their homes; however, that would not explain association with other non-MAC species of NTM. Longitudinal studies with larger numbers are needed to distinguish these possibilities. Therefore, while the current study identified an association, it did not address cause and effect.

Our study had several limitations. As mentioned previously, it is relatively small with 40 matching sets at most per POU source. With a limited sample size, we are not as able to detect more subtle increases in odds ratios, which may be the case for the POU sources other than shower aerosols. Our estimated risk for shower aerosol was higher, and we were able to detect an association in shower aerosols despite such a small study.

Our microbiological and molecular methods may not have detected all NTMs present in the samples, as many plates were lost to mold overgrowth or desiccation. Mycobacteria can be challenging to culture in a lab. The blinding of laboratory personnel to case and control status helped to minimize bias and preferential effort. between case and control samples. We were unable to address potential bias due to collection dates of case and control environmental samples. Due to our recruiting timeline, we recruited and sampled case homes before recruiting and sampling their matched control homes. Recruitment of cases began in January 2009 and ended in January 2011. Sampling of case homes began in February 2009 and ended in February 2011. Recruitment of matched controls began in May 2010 and ended in July 2011. Sampling of matched control homes began in July 2010 and ended in August 2011. If laboratory technique improved over time, personnel would have been better at detecting isolates from control homes than from case homes, which would bias results toward the null and indicate the true association was potentially larger than we measured. If technique worsened due to protocol fatigue, personnel would have been better at detecting isolates from case homes than control homes, which would bias results away from the null and potentially underestimate the true association. Despite this potential bias toward the null, we were able to find an association for shower aerosol NTM colonization and disease.

Our study was not powered to see beyond binary exposure of whether NTM was present or absent. We were unable to divide sources into high-, low-, and no-colonization, and we were unable to see subtle associations that may exist with low colonization.

In summary, we observed that the homes of MAC PD patients have greater detectable NTM colonization in the shower aerosol than the homes of their matched controls. We were unable to detect the association between NTM colonization and MAC PD status for other sites in

the homes (taps, soil). The results of our study, which associates health risk with environmental exposure to opportunistic pathogens, are consistent with past suggestions, based solely on environmental occurrence, that shower aerosols are potentially significant sources of NTM exposure in homes. Future epidemiological investigations should look more at residential exposures, including but not limited to shower aerosols, in the homes of individuals with compromised immune or pulmonary systems. Such studies will help to discern additional potential risk factors in residential environments.

Chapter 3: Aim 2

A. Abstract

Background: Aim 1 showed a significant association between disease and in-home exposure to non-tuberculous mycobacteria (NTM) from shower aerosols. There is a need to better understand what affects the colonization of homes by NTM. Studies looking at bacteria in premise plumbing have not adequately included predictor variables including house and homeowner characteristics, including the socioeconomic status of the homeowners, homeowner's usage of water, homeowner's cleaning and maintenance habits, the age of the house, whether any remodeling or major plumbing job have occurred in the past, the pipe materials and the premise plumbing system.

Objectives: To explore whether house and homeowner characteristics affect the colonization of NTM in homes in water point-of-use (POU) sources.

Methods: This is an exploratory analysis utilizing the previous case-control study from Aim 1 as a cross-sectional study. Environmental samples were collected from bathroom faucets, kitchen faucets, shower aerosols of 107 homes. Mycobacterial load in these samples was quantified by using bacteriological culture combined with PCR. The presence of NTM colonization in each POU source (shower aerosol, bathroom faucet, kitchen faucet) was compared in univariable logistic regressions on all predictor variables followed by a multivariable logistic regression. The magnitude (amount of NTM colonization) in each POU source was also compared in univariable linear regression on all predictor variables followed by a multivariable linear regression.

Results: Associations for the predictor variables relating to house or homeowner characteristics remain uncertain; however, the large effect estimates and wide confidence intervals suggest that more data in a future study would improve precision and potentially be able to better detect associations between the predictor variables related to the house or homeowner, and NTM colonization.

Discussion: This was an exploratory study to see whether house or homeowner characteristics should be included in future studies. We were unable to detect associations due to our limited sample size, the limitations of a secondary analysis, and data quality issues. From a theoretical and practical standpoint, predictor variables relating to the house or homeowner characteristics should be included in future study designs with a qualified technical expert.

B. Introduction

With reports in the scientific and popular press of the typical individual spending as much at 90% of their time indoors (Klepeis et al., 2001; Westervelt, 2012), there is more awareness of the potential health risks inside the built environment. One of the core parts of residential buildings is the plumbing system (premise plumbing). Premise plumbing materials and technology have changed in use and popularity over the decades. Housing codes, which include different requirements for pressure and water technologies, have also changed over the decades. For example, prior to World War II, metal piping was popular until metal was needed for the war effort. Plastic piping, such as polypropylene and polyethylene, first came into use in 1950s but became popular after 1970's.

Piping material for the premise plumbing can include stainless steel, galvanized steel, zinc-galvanized steel, lead, copper, polyvinyl chloride (PVC), pipes, cross-linked polyethylene plastic pipes, and cement. The cost of the piping varies with material as well as labor costs since plastic is more easily manufactured and more easily installed. For example, the plumbing of a typical two-bathroom home could cost as much as $10,000 using copper pipes, but could be as cheap as $4,000 using plastic pipes. Therefore, piping material can be affected by socioeconomic status of the homeowner, as can schedules for replacement of old plumbing and remodeling

Mycobacterium avium complex (MAC) and other non-tuberculous mycobacteria (NTM) adhere to and form biofilms on stainless steel, glass, zinc-galvanized steel, copper, and PVC (Mullis & Falkinham, 2013). Biofilm growth and the general bacterial community structure are affected the premise plumbing materials (Wang et al., 2014). For example, copper is inhibitory to bacterial aggregation and results in less biofilm activity (Liu et al., 2016, Beeton, Aldrich-Wright, & Bolhuis, 2014). In contrast, iron-based materials, including steel pipes, are prone to

bacterial growth promotion due to corrosion products interacting with residual disinfectant, and to their tendency to retain and provide nutrients to biofilms. Data about the effect plastic piping can have on bacterial colonization is mixed. Its polymer additives can promote biofilm growth and bacteria proliferation (Liu et al., 2016, Kilb et al., 2003), but *in vitro* experiments have found that plastic piping results in less biofilm formation (Liu et al., 2016, Kerr et al., 1998, Niquette et al., 2000, Momba &Kaleni, 2002).

Remodeling and the disruption of premise plumbing, including replacement of plumbing materials, can promote intrusion of bacteria into a system. Geography affects the availability of building materials being used, the properties of water distribution systems, and the presence of environmental microbes in the water (Falkinham, 2015). Frequency of shower/bath usage may affect bacterial colonization in the premise plumbing by modulating water retention time and the frequency and intensity of flushing.

Several studies have explored how and why environmental bacteria are detected in the premise plumbing of buildings. Many studies focus just on the diversity of bacteria. For example, a study of the composition of bacteria in shower aerosols, Feazel et al., 2009, did not consider any details of the environment, except for whether the shower water was sourced from a well or a municipal-supplied system.

Past studies of environmental bacteria in premise plumbing of buildings included variables related to water quality (temperature, pH, disinfectant residuals, total oxygen content), type of building (e.g. residential, hospital, community home), piping material, use of in-line filters, location of premise plumbing, water stagnation and residence time, diameter of pipes, and the design of faucets and devices at point-of-use (POU) sources (Li et al., 2018, Miyagi et al., 2017, Bedard et al., 2016, Rhoades et al., 2015, Lu et al., 2014, Lautenschlager et al. 2010).

However, those studies did not include important variables relating to house and homeowner characteristics, including demographics, owner upkeep/maintenance, frequency of usage, when the building was constructed, and whether remodeling occurred.

We need to better understand the influencing factors in premise plumbing which may contribute to bacteria growth in buildings. A better understanding of these factors will help identify areas at higher risk, and in the future help policymakers and homeowners implement effective control measures.

Aim 1 showed a clear association between disease and in-home exposure to NTM from shower aerosols. This supports a need for better understanding of in-home exposure to these organisms. However, assessing the in-home exposure and any contributing factor to bacterial growth in buildings is challenging. The objective of this aim was to ask whether physical or socioeconomic parameters affect mycobacterial colonization of premise plumbing. We hypothesized that the decade of build, remodeling occurrence, pipe material, water heater temperatures, frequency of showerhead replacement, the water distribution system, socio-economic status, geography, and frequency of usage could be associated with colonization by NTM in shower aerosols or tap water. While water quality parameters including disinfection residuals, pH, and oxygen content are also likely to be important, we could not assess them because these data were not measured in the original epidemiology study.

C. Methods

This section of the dissertation continues the previous population-based study (Dirac et al. 2012) associating health risk with environmental exposure to nontuberculous mycobacteria.

This section of the dissertation is a cross-sectional secondary analysis of the same study. Since we found that shower aerosols are a significant POU source, we were interested in examining factors that may affect colonization of NTM in the showerhead and their resulting aerosols. Additionally, since bulk water POU sources, such as water from the bathroom faucet and water from the kitchen faucet run through the same premise plumbing, we were also interested in examining factors which may affect colonization of NTM in the bathroom faucet and kitchen faucet samples.

Study Design and Subject Recruitment

We utilized the data from the original population-based study (Dirac et al., 2012) involving adult HIV negatives subjects and their homes, and the associated microbiological data, as described in Chapter 2. Recruitment spanned from January 2009 to July 2011. Subjects were recruited through hospital systems in Washington and Oregon and random-digit dialing. Excluded from the study were individuals who had cystic fibrosis, HIV, or individuals who lived in nursing homes or similar institutions.

In the current secondary analysis, we included all homes that had environmental sampling completed at 3 POU sources including the kitchen faucet, bathroom faucet, and shower aerosols. This analysis did not differentiate between case and control homes. Therefore, it included a total of 107 individual homes (previously they were identified as 56 cases and 51 control in Aim 1 of the dissertation).

In-home interview

Once study participants were contacted via telephone in the original case-control study (Dirac et al., 2012), they received a home visit that included informed consent, an interview, and collection of multiple environmental samples from five POU sites around the house or apartment (Dirac et al., 2012). Participants were asked questions about their health, their homes, behaviors hypothesized to generate aerosol exposures, demographic data, date of diagnosis (for cases), and where the subject lived on the index date. Questions about their homes included the decade the house was constructed, whether any remodeling occurred, the pipe material, water heater temperatures, frequency of showerhead replacement, and their water distribution system.

More specifically, during the in-home questionnaire (in Appendix B), participants were asked the following questions about the characteristics of their house that they have lived in which may contribute to NTM growth:

- In what decade was the home you lived in built?
- Did this home have its original plumbing?
- What was the name of your water [distribution] system?
- Do you know what temperature of your hot water tank? If yes, what?
- If you turned on the hot water tap in your home and let it run, the water that came out could best be described as warm, hot, or scalding?
- Do you know what material the pipes in that home are made of? If so, what are they?
- When, if ever, did you last change your showerhead?

Environmental Sampling

At the same visit, we also collected environmental samples from 5 POU sources in and around their home. Environmental sampling was conducted as described in Chapter 2; in this analysis we only considered the water-based POU sources: bathroom faucet water, kitchen faucet water, and shower aerosols.

Laboratory processing and data entry

This section of the dissertation follows the methods previously described in chapter 2 (Aim 1) regarding laboratory processing for shower aerosols and bulk water from bathroom and kitchen faucet water samples. Laboratory processing was completed prior to this dissertation as shown in Figure 2.1. More details of the laboratory process and the figure are located in Chapter 2.

In short, primary plating of bulk water samples was accomplished by vacuum filtration and the petri dish in the BioStage® cascade impactor constituted the primary culture plate for shower aerosol samples. After environmental sample were collected, primary cultures were grow and incubated in sealed bags at 37 °C and observed for 8 weeks, or until the culture became desiccated or molded. Each week, new colonies and new colony morphotypes were recorded into a Microsoft Access database by the candidate. Each new colony type was transferred by sterile loop to a new sub-culture plate by streaking for isolation. All sub-culture plates were similarly incubated and observed for 8 weeks or until the culture became desiccated or molded. This process was repeated until sub-culture plates only had a single morphotype and DNA extraction and PCR identification could proceed. DNA was extracted from purified colonies by a boil-prep, heat-lysis method and PCR identification described by Wilton and Cousins (1992). Detected

band sizes in the gel were correlated with genus and species identifications as outlined in Table 2.2. From this, we were able to get a count of identified NTM species per isolate and backtrack it to the individual and/or original POU source to result in a total number of NTM isolates found.

Statistics

Statistical analyses were performed using STATA /SE v. 14.2. We asked whether there are any variables associated to NTM colonization of shower aerosols, bathroom faucets, and kitchen faucets.

The number of positive NTM isolates found in showerhead aerosol samples were examined as it relates to several variables including decade of build, remodeling, geography, race, education, and race. The variables of interest include the following: 1.) decade of build, 2.) whether remodeling occurred, 3.) geography, as defined by state, and by the side of the state (e.g. west and east), 4.) ethnicity/race, 5.) education level (as a proxy for socioeconomic status), and 6.) frequency of shower/bath usage.

Decade of build, while originally a categorical variable by each decade, was collapsed into era of build since the categories are too small and not populated enough. Era of build is divided into the pre-world war II era (1900-1940), before American building materials, including metals were re-distributed to the war effort, the modern era (1980-2000) when plastics and polymers because more popular, and the decade in between (1950-1970).

“Side-of-state” analyses capture many different aspects since the climate and geography of western Washington and western Oregon differ from the those of eastern Washington and eastern Oregon. Western subjects were relatively closer to the coast, and generally more urban. Eastern subjects live in drier climates and were generally more rural.

Race and ethnicity was recorded as categories. However, since the categories were too small and not sufficiently populated, they were collapsed into a binary variable: white/non-Hispanic and other. Education was similarly collected in seven categories but then collapsed into three categories: high school or equivalent; some college, associate's degree, or occupational/vocational degree; and bachelors, masters, professional or doctorate degree holders.

Frequency of shower usage and bath usage originally came from five categories. However, since they were too small as well, they were also collapsed into three categories. The categories were determined in a way to help assure that they were relatively well distributed and not too small. These variables are only included for the shower aerosol analysis.

We considered the outcome variable, NTM colonization in two ways, 1.) as a binary variable with the higher-exposed group defined as having one or more isolates found, and the lower exposed group defined as having zero isolates grown and 2.) as a continuous variable of number of positive NTM isolates found. In this exploratory analysis, we wanted to consider all possible interpretations of the microbiological data.

Categorical exposure variables compared to binary outcome variables (colonization of NTM or none) were analyzed by chi-square. If the exposure variables were binary, they were analyzed by Fisher's exact test. Binary exposure variables include the presence of original piping, state, side of the state, and race. All other exposure variables were categorical. Additionally, one-way ANOVA tests were done on the categorical exposure variables compared to the outcome variable (number of NTM isolates found) as a continuous variable. If the exposure variable was binary, two-tailed t-test were done.

This aim asked whether any of the above variables were associated with any presence of NTM isolates in the water-based POU sources, or rather whether the exposure variables affect whether any colonization happens at all. To determine this, univariable logistic regressions were initially performed on all predictor variables. Multivariable models for each outcome were created using predictor variables that were significant at $\alpha<0.20$ by looking at the p-value of the probability of obtaining the chi-square statistic. Any exposure variables that were found to have a p-value <0.20 were then included in the multivariable model. Significance of the multivariable models was determined at $\alpha <0.05$.

This aim also asked whether any of the exposure variables were associated with heavier presence of NTM in the water-based POU sources, or rather whether the variables affect how contaminated a POU source can be. To determine this, univariable linear regressions were initially performed on all variables. Multivariable models for each outcome were created using variables that were significant at $\alpha<0.20$ by looking at the p-value of the F-test. Any exposure variables that were found to have that p-value were then included in the multivariable model. Significance of the multivariable models was determined at $\alpha<0.05$.

D. Results

The study included 107 individual homes that had shower aerosol samples. This included 56 of the cases and 51 of the controls in the original study (Dirac et al., 2012). Of these 107 homes with shower aerosol samples, 75 (70.1%) had no positive isolates of NTM, 32 (29.9%) had one or more positive isolates.

This analysis also included 108 individual homes that had bathroom faucet samples and kitchen faucet samples. This included 55 of the cases and 53 of the controls in the original study

(Dirac et al., 2012). Of these bathroom samples, 59 homes (54.6%) had no positive isolates and 49 homes (45.3%) had 1 or more positive isolates. Of the kitchen samples, 58 homes (53.7%) has no positive isolates, and 50 homes (46.3%) had 1 isolate or more. This distribution is summarized in Figure 3.1.

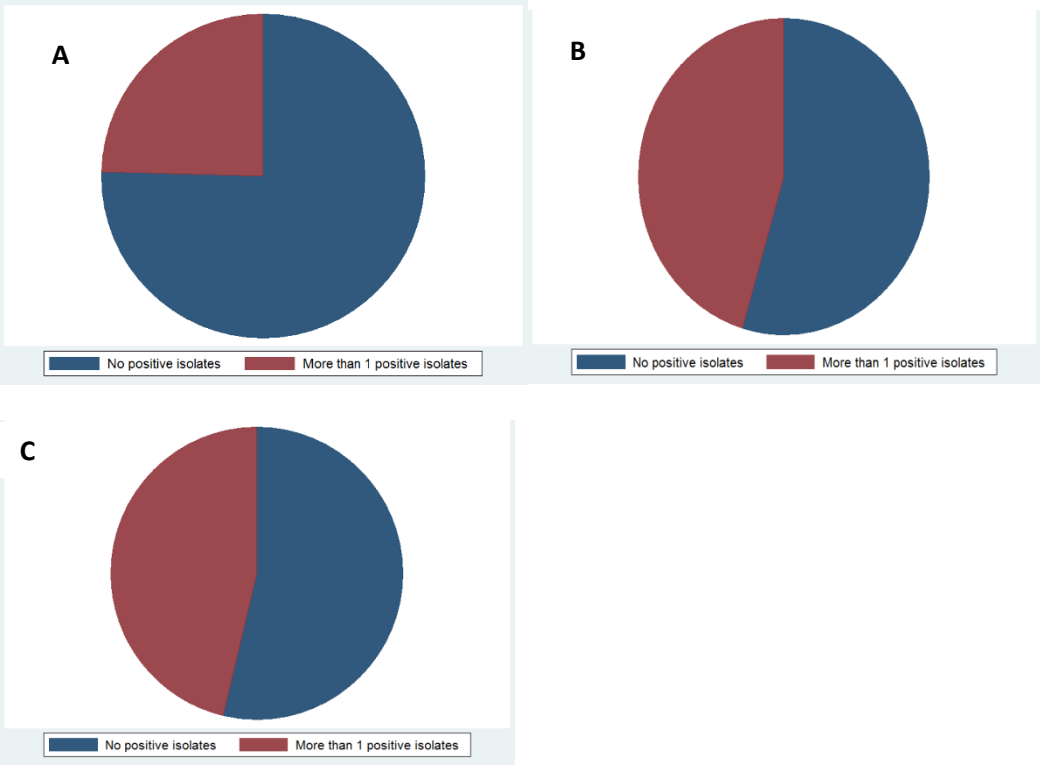

Figure 3.1 Number of Positive NTM Isolates found in shower aerosols (A), bathroom faucet (B), and kitchen faucet (C).

Variables of interest for shower aerosols, bathroom faucet, and kitchen faucets are summarized in Tables 3.1, 3.2, and 3.3 respectively. The majority of the homes sampled had their original piping, were from the western side of Washington, and their homeowners were mostly non-Hispanic whites.

Table 3.1 Summary of exposure variables for presence of NTM in shower aerosols. P-values were determined by chi-square.

Variable	Count of Houses with no isolates positive for NTM, N = 75	Count of Houses with at least 1 isolate positive for NTM, N = 32	P-value
Built Era			0.62
Pre WWII (1900-1940)	13	8	
Pre-Plastic Boom (1950-1970)	31	11	
Modern (1980-2000)	31	13	
Has original piping			0.35
No	18	11	
Yes	55	21	
Geography by State			0.36
OR	24	7	
WA	51	25	
Geography by Side			1.00
East	4	1	
West	71	31	
Race/Ethnicity			0.20
White, Non-Hispanic	71	28	
Other	3	4	
Black, Non-Hispanic	0	1	
Asian or Pacific Islander, Non-Hispanic	1	1	
Native American, Non-Hispanic	1	0	
Hispanic	0	2	
Other	2	0	
Education			0.34
High School or equivalent	10	5	
Some college, Associates, or Occupational	29	8	
Bachelors or higher	34	19	
Bachelors	16	8	
Masters	9	8	
Professional or Doctorate	9	3	

Bath or Shower user			0.80
Exclusively Showers	28	14	
Mostly Showers	28	11	
Bathes and Showers at similar frequency	8	3	
Mostly or Exclusively Baths	8	4	
Both baths and showers infrequently	3	0	
Frequency of Shower Usage			0.36
No more than 1 time	11	4	
Several times a week	18	12	
Daily	46	16	
Frequency of Bath Usage			0.85
Never	29	14	
At least 1 time a week	30	11	
More than 1 time a week	16	7	

Table 3.2 Summary of exposure variables for presence of NTM in bathroom faucets. P-values were determined by chi-square for categorical variables.

Variable	Count of Houses with no isolates positive for NTM, N = 59	Count of Houses with at least 1 isolate positive for NTM, N = 49	P-value
Built Era			0.61
Pre WWII (1900-1940)	12	9	
Pre-Plastic Boom (1950-1970)	21	22	
Modern (1980-2000)	26	18	
Has original piping			1.00
No	15	13	
Yes	43	35	
Geography by State			0.67
OR	16	16	
WA	43	33	
Geography by Side			0.66
East	2	3	
West	57	46	
Race/Ethnicity			0.22
White, Non-Hispanic	54	47	
Other	5	1	
Black, Non-Hispanic	1	0	
Asian or Pacific Islander, Non-Hispanic	2	0	
Native American, Non-Hispanic	1	0	
Hispanic	1	1	
Education			0.59
High School or equivalent	9	6	
Some college, Associates, or Occupational	22	14	
Bachelors or higher	28	27	
Bachelors	12	12	
Masters	9	9	
Professional or Doctorate	7	6	

Table 3.3. Summary of exposure variables for presence of NTM in kitchen faucets. P-values were determined by chi-square.

Variable	Count of Houses with no isolates positive for NTM, N = 58	Count of Houses with at least 1 isolate positive for NTM, N = 50	P-value
Built Era			0.71
Pre WWII (1900-1940)	10	11	
Pre-Plastic Boom (1950-1970)	23	21	
Modern (1980-2000)	25	18	
Has original piping			0.56
No	14	15	
Yes	42	35	
Geography by State			0.73
OR	18	14	
WA	40	36	
Geography by Side			0.12
East	1	4	
West	57	46	
Race/Ethnicity			0.46
White, Non-Hispanic	53	47	
Other	5	2	
Black, Non-Hispanic	0	1	
Asian or Pacific Islander, Non-Hispanic	2	0	
Native American, Non-Hispanic	1	0	
Hispanic	1	1	
Other	1	0	
Education			0.14
High School or equivalent	9	5	
Some college, Associates, or Occupational	24	13	
Bachelors or higher	25	30	
Bachelors	12	12	
Masters	6	12	
Professional or Doctorate	7	6	

For the univariable logistic regression, the findings are summarized in Table 3.4, which shows only those predictor variables that were significant at $\alpha < 0.2$. In these analyses, the outcome, NTM colonization was considered as a binary variable. For the analysis of kitchen samples, two variables, state side, and education of homeowner were able to progress to the multi variable logistic regression. Shower aerosols, and bathroom analyses each found only one variable, race, to have a p-value below 0.2. For the analysis of shower aerosols and bathroom samples, we were not able to progress to the multi variable logistic regression.

In the multivariable logistic regression (Table 3.5), we found no significant associations between NTM isolates from the kitchen faucet with side-of-state and education. The eastern side of the state had a high odds ratio but also had a wide confidence interval (OR, 7.18, 95% CI 0.67, 77.39).

Summaries of the exposure variables of interest compared to NTM colonization as a continuous variable, including the two-tailed t-test and one-way ANOVA analyses for shower aerosols, bathroom faucet, and kitchen faucets are shown in Tables 3.6, 3.7, and 3.8 respectively.

Table 3.4 Summary of univariable logistic regressions of NTM presence or absence in shower aerosol, bathroom faucet, and kitchen faucet samples, only variables with α=0.2

POU	Variable	Odds ratio	95% confidence interval	P-value
Shower Aerosols				
	Race/Ethnicity			0.13
	White, Non-Hispanic	Referent	--	
	Other	3.38	0.71, 16.08	
Bathroom	**Race/Ethnicity**			0.13
	White, Non-Hispanic	Referent	--	
	Other	0.23	0.03, 2.04	
Kitchen	**Side-of-State**			0.11
	West	Referent	--	
	East	4.96	0.54, 45.89	
	Education			0.14
	Bachelors or higher	Referent	--	
	Some college	0.45	0.19, 1.07	
	HS	0.46	0.14, 1.56	

Table 3.5 Multivariable logistic regression of kitchen samples for NTM colonization.

Variable		Odds ratio	95% Confidence interval
Side-of-State			
	West	Referent	--
	East	7.18	0.67, 77.39
Education			
	Bachelors or higher	Referent	--
	Some college	0.42	0.17, 1.03
	HS	0.46	0.13, 1.61

Table 3.6 Summary of exposure variables for number of NTM isolates found in shower aerosols. P-values were determined by one-way ANOVA for categorical variables, or by two-sample t-test for binary variables.

Variable	N	Mean	SD	P-value
Built Era				
Pre WWII (1900-1940)	21	5.38	9.22	0.86
Pre-Plastic Boom (1950-1970)	42	4.05	9.28	
Modern (1980-2000)	44	5.86	15.69	
Has original piping				
No	29	7.38	17.24	0.29
Yes	76	4.50	9.88	
Geography by State				
OR	31	4.65	1.86	0.77
WA	76	5.42	13.01	
Geography by Side				
East	5	2.00	2.00	0.55
West	102	5.35	12.48	
Race/Ethnicity				
White, Non-Hispanic	99	5.07	12.36	0.59
Other	7	7.71	11.76	
Education				
High School or equivalent	15	8.60	22.18	0.51
Some college, Associates, or Occupational	37	4.22	10.80	
Bachelors or higher	53	5.11	9.32	
Bath or Shower user				
Exclusively Showers	42	5.60	14.25	0.64
Mostly Showers	39	6.72	13.19	
Bathes and Showers at similar frequency	**11**	1.00	2.41	
Mostly or Exclusively Baths	12	4.00	6.66	
Both baths and showers infrequently	3	0.00	0.00	
Frequency of Shower Usage				
No more than 1 time	15	3.20	6.13	0.29
Several times a week	30	8.13	17.64	
Daily	62	4.26	9.87	
Frequency of Bath Usage				
Never	43	5.47	14.11	0.48
At least 1 time a week	41	6.39	12.93	
More than 1 time a week	23	2.57	5.21	

Table 3.7 Summary of exposure variables for number of NTM isolates found in bathroom faucets. P-values were determined by one-way ANOVA for categorical variables, or by two-tailed t-test for binary variables.

Variable		N	Mean	Std Dev	P-value
Built Era					0.41
	Pre WWII	21	6.48	9.17	
	Pre-Plastic Boom	43	11.12	14.88	
	Modern	44	11.46	16.67	
Has original piping					
	Yes	78	10.89	15.24	0.62
	No	28	9.25	14.00	
Geography by State					
	WA	76	9.70	14.41	0.48
	OR	32	11.91	15.71	
Geography by Side					
	West	103	9.69	13.92	0.03
	East	5	24.00	25.41	
Race/Ethnicity					
	White, Non-Hispanic	101	10.94	15.07	0.14
	Other	6	1.67	4.08	
Education					
	High School or equivalent	15	9.60	14.40	0.12
	Some college or Associates	36	6.56	10.13	
	Bachelors or higher	55	13.15	17.12	

Table 3.8 Summary of exposure variables for number of NTM isolates found in kitchen faucets. P-values were determined by one-way ANOVA for categorical variables, or by two-tailed t-test for binary variables.

Variable	N	Mean	SD	P-value
Built Era				
Pre WWII	21	12.95	20.78	0.76
Pre-Plastic Boom	44	13.02	18.41	
Modern	43	10.35	16.73	
Has original piping				
Yes	77	11.62	2.04	0.62
No	29	13.62	3.58	
Geography by State				
WA	76	10.50	1.88	0.20
OR	32	15.38	3.83	
Geography by Side				
West	103	11.44	1.75	0.19
East	5	22.40	10.99	
Race/Ethnicity				
White, Non-Hispanic	100	12.11	17.92	0.89
Other	7	11.13	8.77	
Education				
High School or equivalent	14	9.86	18.00	0.31
Some college or Associates	37	9.14	15.54	
Bachelors or higher	55	14.75	19.84	

For the univariable linear regressions, the findings are summarized in Table 3.9. Table 3.9 shows only those exposure variables that were significant at $\alpha < 0.2$. Only the bathroom faucet analyses were able to progress to the multi variable logistic regression since three variables, side-of-state, education of homeowner, and race of homeowner as a binary variable achieved significance. Kitchen found only 1 variable, side as a binary variable, to be significant at that level. No analyses for the shower aerosols were found to be significant at $\alpha<0.2$.

Table 3.10 displays the findings of the multivariable linear regression to predict the level of NTM colonization in bathroom faucet samples based on the side of the state, the race of the homeowner, and the education level of the homeowner. For the side-of-state analysis, we see the East has a large coefficient but again a large confidence interval which includes 1.

Table 3.9 Summary of univariable linear regressions for shower aerosol, bathroom faucet, and kitchen faucet samples, only variables with α=0.2

POU	Variable	Coefficient	Std Error	95% CI	P-value
Kitchen	**Side**				0.19
	West	Referent			
	East	10.96	8.27	-5.44, 27.36	
Bathroom	**Side**				0.03
	West	Referent			
	East	14.31	6.65	1.13, 27.50	
	Race				0.14
	White	Referent			
	Other	-9.27	6.19	-21.55, 3.00	
	Education				0.12
	High School or equivalent	-3.55	4.29	-12.05, 4.96	
	Some college, Associates, or Occupational	-6.59	3.16	-12.85, -0.33	
	Bachelors or higher	Referent			

Table 3.10 Multivariable linear regression for bathroom faucet samples.

Variable		Coefficient	Std Error	95% CI
Side				
	West	Referent		
	East	13.78	6.63	0.62, 26.94
Race				
	White	Referent		
	Other	-8.55	6.06	-20.57, 3.48
Education				
	High School or equivalent	-6.60	3.09	-12.72, -0.47
	Some college, Associates, or Occupational	-2.69	4.21	-11.04, 5.66
	Bachelors or higher	Referent		

E. Discussion

While there is a lot of published research looking at the in-home environment and potential sources of exposure to pathogenic environmental mycobacteria, few have considered the variables we investigated in our analysis for Aim 2: 1.) decade of build, 2.) whether remodeling occurred, 3.) geography, as defined by state, and by the side of the state (e.g. west and east), 4.) ethnicity/race, 5.) education level (as a proxy for socioeconomic status), and 6.) frequency of shower/bath usage.

Variables including the pipe material, water heater temperatures, frequency of showerhead replacement, their water distribution system were collected in the course of the original study. However, concerns about data quality complicated further analyses. We found that homeowners were not sure about their pipe materials, gave wildly implausible temperatures for their water heaters, or were not certain about their water distribution systems. For example, the default water heater temperature is typically set at 140 degrees, but subjects reported temperatures as high as 220 degrees.

We conducted many statistical tests for Aim 2, increasing the risk of Type 1 error. Since the probability of Type 1 error increases with multiple tests, we cannot over interpret our results. We found some suggestions of associations with large effect sizes but wide confidence intervals. Our study was relatively small with only 107 houses. Additionally, some categories, such as side-of-state were not equally sampled. These problems arose in part from the fact that this was a secondary analysis of a case-control study. For example, the number of people in the eastern side of the two states in our study were relatively low, such that our analysis is based on only five houses. Associations for all the predictors remain uncertain, but the large effect estimates and

wide confidence intervals suggest that more data in a future study would improve precision and potentially be able to better detect associations between the predictor variables related to the house or homeowner, and NTM colonization.

Other variables were used as proxies for dropped house characteristics. For example, the decade of home construction is a less-than-perfect proxy for plumbing material. Year of construction relates to many other variables, including pipe material and may be better included as an interaction variable. If we were to only have good quality data for year of construction but not for any of the intermediates in its causal pathway, we can only speculate about the pathway. If we were able to include not only the year of construction but also intermediate variables of interest in the model, we would be able to interpret year without the mediation of the other variables in the model. Presence of a significant interaction would indicate that the effect of one predictor variable on the response variable is different at different values of the other predictor variable. For example the source and composition of steel pipes used can differ from year to year and could result in different effects on the colonization of bacteria. Other variables may serve as proxies for socioeconomic status which may affect the type of piping material used in the home.

Future studies looking at home exposure to environmental bacteria should carefully measure variables including the age of the house, whether any remodeling or major plumbing job have occurred in the past, the socioeconomic status of the homeowners, homeowner's usage of water, and the homeowner's cleaning and maintenance habits,. Ideally, they should also measure variables relating to the pipe material and the premise plumbing system, as assisted by qualified technical experts. The original study may have been improved by the inclusion of built environment experts. In-home exposures and exploring what factors may contribute to bacteria

colonization is complicated and would benefit from interdisciplinary teams consisting of not only environmental health or epidemiology specialists, but also a qualified technical specialist to accompany on sampling or in-home visits.

Chapter 4: Discussion and Conclusions

In this study, we investigated residential sources of exposure to non-tuberculous mycobacteria (NTM). NTM are opportunistic pathogens known to persist in the environment and cause pulmonary disease. Previous case-only studies identified potential sources of infection in homes, including but not limited to shower aerosols. However, the extent to which specific types of household exposure relate to pulmonary disease was not known.

Aim 1 used a population-based matched case-control design to determine if exposure to NTM from various aerosolizing sources in the home is associated with MAC (*Mycobacterium avium* complex) pulmonary disease in a generalizable population. We hypothesized that the homes of those who are clinically diagnosed with MAC pulmonary disease (MAC PD) have higher MAC and other NTM colonization than the homes of age, gender, and geographically matched controls and conducted a case-control study. We were able to provide quantitative evidence to support previous conjectures that shower aerosols are potentially significant sources of exposure and fill a gap in the existing NTM pulmonary disease (NTM PD) epidemiology literature. To our knowledge, this is the first observational study reporting an association between human health and exposure to NTM in the residential environment. With regard to other point-of-use (POU) sources, we were unable to detect the associations in our current study and the associations remain uncertain. While odds ratios were consistently greater than one, wide confidence intervals limit the conclusions we can draw without further data.

Aim 2 explored aspects of house and homeowner characteristics that are not commonly included in epidemiological studies of opportunistic pathogens like NTM in residential buildings. NTM are able to persist and recolonize in the built environment. Previous environmental studies

that sampled water in the home have not considered many aspects of the homeowner and the built environment, such as demographic information of the homeowner, maintenance, frequency of usage of POU sources, age of the building, and whether any remodeling or major plumbing job occurred in the past. While we were unable to detect associations between NTM colonization and these variables, there may nonetheless be associations that our small cross-sectional study was unable to detect. There are many additional variables of scientific interest that we were unable to incorporate in our analysis, including the pipe material, water temperature, details about the premise plumbing system and water distribution system, and frequency of showerhead replacement due to data quality issues. All of the above-mentioned variables theoretically can indirectly or directly affect environmental bacterial growth in the homes, and should be included in studies large enough to find such associations if they exist. There is a need for concepts of the built environment to be incorporated into environmental health and epidemiology research looking at in-home exposures. Future studies looking at in-home exposures should take care to assemble not only environmental health or epidemiology specialists, but also qualified technical experts, to ensure good data quality when collecting and measuring these predictors on site.

The dissertation continued a case-control study to: 1) determine if exposure to NTM or MAC from POU sources in the home is associated with MAC PD, and 2) explore whether different house or homeowner predictors associate with NTM colonization. In general, the study utilized a culture-dependent, 1-time sampling scheme in 107 houses. This method was sufficient to answer Aim 1 for shower aerosol sources. After data collection on the original study, culture independent methods became more utilized, accessible, sensitive, and more able to detect NTM accurately. While we were unable to include culture-independent methods in this dissertation, future studies should consider including them in their study. While culture independent methods

will not be able to answer whether the bacteria of interest are active and viable, they are still sufficiently able to answer whether the presence of the bacteria associates with disease in POU sources in the home. Sampling methods for POU sources other than shower aerosols are less defined and may need to be optimized for NTM detection for future studies of in-home exposure to other POU sources. In general, future study designs should focus on: 1) optimizing bacteria sampling and detection in other POU sources or in-home exposure, and 2) exploring whether other POU sources in the home associate with disease. Future studies should adequately measure and collect, with a trained technical expert, all relevant predictors that would affect NTM colonization in homes, including but not limited to pipe material, water distribution system details, details of the premise plumbing (e.g., location of piping, temperature of water heater) water quality parameters (e.g., disinfectant residuals, temperature, oxygen content, pH), age of the building, occurrence of any remodeling or major plumbing repairs, homeowner demographic information (socioeconomic status, race, education, geography), and homeowner maintenance habits (e.g., frequency of changing showerhead).

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Appendices

List of Appendices

A. Age Categories
B. Except from original Data Collection Form (v7.3 12 May 2009) by M. Ashworth Dirac

Appendix A. Age Categories

Case age	Lower		Upper	Case age	Lower		Upper
18	18	-	23	60	53	-	67
19	18	-	24	61	54	-	68
20	18	-	25	62	55	-	69
21	18	-	26	63	56	-	70
22	18	-	27	64	57	-	71
23	18	-	28	65	58	-	72
24	19	-	29	66	59	-	73
25	20	-	30	67	60	-	74
26	21	-	31	68	60	-	75
27	22	-	32	69	60	-	76
28	23	-	33	70	60	-	80
29	24	-	34	71	61	-	81
30	25	-	35	72	62	-	82
31	26	-	36	73	63	-	83
32	27	-	37	74	64	-	84
33	28	-	38	75	65	-	85
34	29	-	39	76	65	-	86
35	30	-	40	77	65	-	87
36	31	-	41	78	65	-	88
37	32	-	42	79	65	-	89
38	33	-	43	80	65	-	95
39	34	-	44	81	66	-	96
40	35	-	45	82	67	-	97
41	36	-	46	83	68	-	98
42	37	-	47	84	69	-	99
43	38	-	48	85	70	-	100
44	39	-	49	86	71	-	101
45	40	-	50	87	72	-	102
46	41	-	51	88	73	-	103
47	42	-	52	89	74	-	104
48	43	-	53	90	75	-	105
49	44	-	54	91	76	-	106
50	45	-	55	92	77	-	107
51	46	-	56	93	78	-	108
52	47	-	57	94	79	-	109
53	48	-	58	95	80	-	110
54	49	-	59	96	81	-	111
55	50	-	60	97	82	-	112
56	51	-	61	98	83	-	113
57	52	-	62	99	84	-	114
58	53	-	63				
59	53	-	64				

Appendix B. Excerpt from Data Collection v7.3 12 May 2009 by M. Ashworth Dirac
Subject ID#: [IndividualID]

II. Water Use - *One year prior*

The first set of questions is about the ways that you used water in [1year]. For most of the questions, I will ask whether you did a certain activity ever during that year, and then I'll ask about how often you did that activity. Here is a list of the answer options that you can use to answer these questions.

In the year [1year], did you engage in the following activities? If yes, how often?

N= No (never). If "Yes", how often:

A- At least once, but less than once a month
B- About once a month
C- More than once a month, but less than once a week
D- About once a week
E- More than once a week, but less than every day
F- Daily

(*If the subject asks about whether they should describe seasonal variation, simply reiterate that you want to know "about how often you did these things during [1year]", however they interpret that. Some will give frequency during summer months, some will give frequency during winter months, and some will average. If they spontaneously give multiple answers for different times of year, ask them to give a single answer to the question "about how often during [1year]"*.)

q1. [Show1] Showering? N A B C D E F

q2. [Jac1] Using a whirlpool bath, hot tub, or Jacuzzi (with jets on)? N A B C D E F

q3. [Bath1] Taking a bath (in a regular bathtub or other tub without jets on)? N A B C D E F

q4. [Sauna1] Using a sauna or steam room? N A B C D E F

q5. [Humid1] Using a humidifier? N A B C D E F

q6. [Hand1] Hand-washing dishes? N A B C D E F

q7. [Washer1] Using a dishwasher? N A B C D E F

This refers to actually running the washer, not opening and closing multiple times to add dishes to a single load.

q8. [Pool1] Going in a swimming pool? N A B C D E F

If yes, q214. [KindP1] What kind of pool? Indoor / Outdoor / Both Indoor and Outdoor

q9. [Hose1] Spraying plants or garden with a hose? N A B C D E F

If yes, q10. [H1_Source] Did the water to the hose come from the same source as household tapwater? Y/N

q11. [Bottle1] Spraying plants or garden from a spray-bottle? N A B C D E F

q12. [Can1] Watering plants or garden from a watering can, bucket, or bottle? N A B C D E F

q13. [Sprink1] Spending time outside (a ½ hour or more) while sprinklers were running? N A B C D E F

q14. [AC1] During [1year], did you use an air conditioner at home? Y/N

If yes, q15. [AC_Months1] How many months of the year? _____

q16. [AC_Type1] What kind? _____

Subject ID#: [IndividualID]

III. Water Use - *Five years prior*

The second set of questions is about the ways that you used water in [5year]. For most of the questions, I will ask whether you did a certain activity ever during that year, and then I'll ask about how often you did that activity. Please use the same list of answer options that you can used for the last section.

In the year [5year], did you engage in the following activities? If yes, how often?

N= No (never). If "Yes", how often:

A- At least once, but less than once a month
B- About once a month
C- More than once a month, but less than once a week
D- About once a week
E- More than once a week, but less than every day
F- Daily

(If the subject asks about whether they should describe seasonal variation, simply reiterate that you want to know "about how often you did these things during [5year]", however they interpret that. Some will give frequency during summer months, some will give frequency during winter months, and some will average. If they spontaneously give multiple answers for different times of year, ask them to give a single answer to the question "about how often during [5year]".)

q17. [Show5] Showering? N A B C D E F

q18. [Jac5] Using a whirlpool bath, hot tub, or Jacuzzi (with jets on)? N A B C D E F

q19. [Bath5] Taking a bath (in a regular bathtub or other tub without jets)? N A B C D E F

q20. [Sauna5] Using a sauna or steam room? N A B C D E F

q21. [Humid5] Using a humidifier? N A B C D E F

q22. [Hand5] Hand-washing dishes? N A B C D E F

q23. [Washer5] Using a dishwasher? N A B C D E F

This refers to actually running the washer, not opening and closing multiple times to add dishes to a single load.

q24. [Pool5] Going in a swimming pool? N A B C D E F

If yes, q215. [KindP5] What kind of pool? Indoor / Outdoor / Both Indoor and Outdoor

q25. [Hose5] Spraying plants or garden with a hose? N A B C D E F

If yes, q26. [H5_Source] …did the water to the hose come from the same source as household tapwater? Y/N

q27. [Bottle5] Spraying plants or garden from a spray-bottle? N A B C D E F

q28. [Can5] Watering plants or garden from a watering can, bucket, or bottle? N A B C D E F

q29. [Sprink5] Spending time outside (a ½ hour or more) while sprinklers were running? N A B C D E F

q30. [AC5] During [5year], did you use an air conditioner at home? Y/N

If yes, q31. [AC_Months5] …how many months of the year? _____

q32. [AC_Type5] …what kind? _____

Subject ID#: [IndividualID]

IV. Water System - *One year prior*

Think of where you lived during the year [1year]. The third set of questions is about the water in your home in the year [1year]. If the answers changed over the course of the year, please give the right answer for most of the months of the year.

q33. [Built1] What decade was the home you lived in in [1year] built?
2000/1990/1980….1910/1900/Before 1900/DK

q34. [Plumb1] Did this home have its original plumbing? Y/N/DK

q35. [Well1] Did you get your water from a well? (*Accept "yes" answers for private wells owned by subject or wells owned by other entities, but accept "no" answers even from those who name a water system that maintains a small well, such as RV partks.*) Y/N/DK

If yes,

q36. [Well_Tx1] …did you (*personally*) treat your well water? Y/N/DK

If yes,

q37. [Tx_Type1] …how? Filtration/Disinfection/Both/Other (_____)

q38. [H2O_Sys1] …what was the name of your water system? _____

q39. [H2O_Tem1] Do you know what temperature your hot water tank was maintained at? Y/N

If yes,

q40. [Temp1] … what? _____ Fahrenheit

q41. [Tem_Qual1] If you turned on the hot water tap in your home and let it run, the water that came out could best be described as (circle one): Warm Hot Scalding

q42. [Know_Pipes1] Do you know what material the pipes in that home were made of? Y/N

If yes,

> q43. q44. [Pipe_Mat1] ([Pipe_Spec1]) …what? Metal (Lead/Copper/Black Steel/Galvanized Steel/Ductile Iron/Bronze/DK)/Plastic (PVC/CPVC/PE/PEX/PB/ABS/DK)

q45. [Feature1] Did that home or garden contain a fountain, artificial pond, artificial stream, or other “water feature”? Y/N/DK

Did your household employ any of the following water filtration methods in [1year]? If yes, how often was the filter changed?

q46. [InlineK1] In-line water-filter for kitchen tap? Y/N/DK

q47. [K1_Freq] Changed every: <3mo 3-6mos >6mos DK

q48. [InlineB1] In-line water-filter for bathroom tap? Y/N/DK

q49. [B1_Freq] Changed every: <3mo 3-6mos >6mos DK

q50. [InlineS1] In-line water-filter for showerhead? Y/N/DK

q51. [S1_Freq] Changed every: <3mo 3-6mos >6mos DK

q52. [InDoor1] Filtered drinking water dispenser in door of refrigerator? Y/N/DK

q53. [D1_Freq] Changed every: <3mo 3-6mos >6mos DK

q54. [Pitcher1] Filtered pitcher (Brita-type) for drinking water? Y/N/DK

q55. [P1_Freq] Changed every: <3mo 3-6mos >6mos DK

Subject ID#: [IndividualID]

V. Water System - *Five year prior*

Think about where you lived during the year [5year]. The fourth set of questions is about the water in your home in the year [5year]. If the answers changed over the course of the year, please give the right answer for most of the months of the year.

q57. [Built5] What decade was the home you lived in in [5year] built?
2000/1990/1980….1910/1900/Before 1900/DK

q58. [Plumb5] Did this home have its original plumbing? Y/N/DK

q59. [Well5] Did you get your water from a well? (*Accept "yes" answers for private wells owned by subject or wells owned by other entities, but accept "no" answers even from those who name a water system that maintains a small well, such as RV partks.*) Y/N/DK

If yes,

q60. [Well_Tx5] …did you (*personally*) treat your well water? Y/N/DK

If yes,

q61. [Tx_Type5] …how? Filtration/Disinfection/Both/Other (_____)

q62. [H2O_Sys5] …what was the name of your water system? _____

q63. [H2O_Tem5] Do you know what temperature your hot water tank was maintained at? Y/N

If yes,

q64. [Temp5] … what? _____ Fahrenheit

q65. [Tem_Qual5] If you turned on the hot water tap in your home and let it run, the water that came out could best be described as (circle one): Warm Hot Scalding

q66. [Know_Pipes5] Do you know what material the pipes in that home were made of? Y/N

If yes,

> q67. q68. [Pipe_Mat5] ([Pipe_Spec5]) …what? Metal (Lead/Copper/Black Steel/Galvanized Steel/Ductile Iron/Bronze/DK)/Plastic (PVC/CPVC/PE/PEX/PB/ABS/DK)

q69. [Feature5] Did that home or garden contain a fountain, artificial pond, artificial stream, or other “water feature”? Y/N/DK

Did your household employ any of the following water filtration methods in [5year]? If yes, how often was the filter changed?

q70. [InlineK5] In-line water-filter for kitchen tap? Y/N/DK

q71. [K5_Freq] Changed every: <3mo 3-6mos >6mos DK

q72. [InlineB5] In-line water-filter for bathroom tap? Y/N/DK

q73. [B5_Freq] Changed every: <3mo 3-6mos >6mos DK

q74. [InlineS5] In-line water-filter for showerhead? Y/N/DK

q75. [S5_Freq] Changed every: <3mo 3-6mos >6mos DK

q76. [InDoor5] Filtered drinking water dispenser in door of refrigerator? Y/N/DK

q77. [D5_Freq] Changed every: <3mo 3-6mos >6mos DK

q78. [Pitcher5] Filtered pitcher (Brita-type) for drinking water? Y/N/DK

q79. [P5_Freq] Changed every: <3mo 3-6mos >6mos DK