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Nontuberculous Mycobacteria in Drinking Water Systems – The Challenges of Characterization and Risk Mitigation

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Abstract

Nontuberculous mycobacteria (NTM) pulmonary infections are a growing concern worldwide, with a disproportionate incidence in persons with pre-existing health conditions. NTM have frequently been found in municipally-treated drinking water and building plumbing, leading to the hypothesis that an important source of NTM exposure is drinking water. The identification and quantification of NTM in environmental samples are complicated by genetic variability among NTM species, making it challenging to determine if clinically-relevant NTM are present. Additionally, their unique cellular features and lifestyles make NTM and their nucleic acids difficult to recover. This review highlights recent work focused on quantification and characterization of NTM and on understanding the influence of source water, treatment plants, distribution systems, and building plumbing on the abundance of NTM in drinking water.

Graphical Abstract

Keywords

Nontuberculous mycobacteria; MAC; *Mycobacterium avium*; opportunistic pathogens; drinking water

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Introduction

Infections due to opportunistic pathogens (OPs) are a growing public health concern. OPs generally only infect susceptible persons, including those with pre-existing health conditions and the immunocompromised. Like other water-associated OPs such as *Legionella pneumophila* and *Pseudomonas aeruginosa*, nontuberculous mycobacteria (NTM) are present in the environment and can proliferate in drinking water systems. NTM possess waxy, “acid-fast” cell walls, which render them more hydrophobic and might allow them to be more readily aerosolized than other bacteria [1]. Sometimes called “biofilm pioneers”, NTM can attach to a variety of surfaces and establish biofilms, and are among select bacteria with the ability to enter and survive within amoebae [1,2]. This combination of properties confers the resistance needed to survive conventional water treatment and proliferate in drinking water systems despite the presence of disinfectant residuals [1].

NTM predominately cause pulmonary infections but also cause skin, soft tissue, and post-operative infections [3–6]. A 2012 study estimated the annual cost of hospitalizations in the U.S. due to pulmonary NTM infections to be \$194 million [7]. NTM infection prevalence has increased over the last two decades. Specifically, positive specimen reporting rates in four U.S. states increased from 8.2 to 16 per 100,000 persons from 1994 to 2014, and rates in England, Wales, and Northern Ireland rose from 0.9 to 7.6 per 100,000 persons from 1995 to 2012 [8–10]. However, the true prevalence of NTM infection is unknown and challenging to determine due to the lack of reporting requirements and difficulties with NTM identification from clinical specimens.

Despite the health and economic importance of NTM infections, little is known about specific sources of human exposure to NTM. Recent work has found NTM in drinking water and water system biofilms, suggesting that contact with drinking water might be one source of pulmonary infections [11,12]. However, substantial knowledge gaps, including the lack of risk assessment models, difficulty in evaluating mechanisms of exposure, and host-specific factors that influence susceptibility, have made it difficult to link NTM infections to drinking water and develop mitigation strategies. Given the recent increases in NTM infections, it is paramount that we:

- a. understand the sources and routes of NTM exposure;
- b. identify the risk factors associated with NTM infections; and
- c. develop risk mitigation strategies to reduce NTM infection.

This review focuses on recent efforts to characterize NTM transfer from natural environments to human hosts through drinking water.

Identification of NTM and Their Characteristics

The genus *Mycobacterium* consists of more than 170 species [13–15]. The vast majority of these species comprise the so-called “nontuberculous mycobacteria”, of which only a few account for most human NTM infections [8,16]. Examples of NTM often associated with infection are the *Mycobacterium avium* complex (MAC, which includes *M. avium*,

Mycobacterium intracellulare, and *Mycobacterium chimaera*), the *Mycobacterium abscessus* complex (MAB, which includes *M. abscessus* subsp. *massiliense*, *M. abscessus* subsp. *abscessus*, and *M. abscessus* subsp. *bolletii*), *Mycobacterium fortuitum*, *Mycobacterium kansasii*, and *Mycobacterium chelonae* [13,16–18]. Identification of NTM is crucial for infection diagnosis, prevention, and risk assessment. NTM identification strategies include culturing with NTM-specific media and use of methods that target NTM-specific proteins, lipids, or nucleic acids (Table 1). Among these methods, nucleic-acid based approaches are most commonly used and employ extraction protocols with rigorous physical and enzymatic steps to lyse the waxy NTM cell wall [19,20]. Sequencing the 16S rRNA gene, which is commonly used for bacterial identification, often does not allow for identification of NTM to the species, subspecies, or strain levels [15,18]. NTM identification beyond the species level is particularly important for MAC and MAB members, as certain subspecies within these groups are associated with distinct clinical outcomes [18,21]. Though sequencing whole genomes or several genes is often necessary for species or strain level resolution, some single genes (*rpoB* and *hsp65*) can provide species resolution depending on the specific sequence site and length. These techniques have been used to identify similar species and strains of NTM within clinical and water system samples collected in the same studies [22,23]. Figure 1 shows the taxonomic relatedness of NTM species recently isolated from human respiratory tract and drinking water samples using *rpoB* sequence analysis.

NTM in Source Waters Used for Drinking Water Production

Regional clustering of pulmonary NTM infections has led to investigations of links between NTM infection and geographic and environmental factors, including characteristics of source waters used for drinking water production. An analysis of NTM infections in Medicare patients in the U.S. found 55 counties in eight states with clusters of infection, and the two counties with the highest risk were located in Louisiana and Hawaii [24]. Geographic factors that correlate with elevated NTM infection rates include higher evapotranspiration rates, a greater percentage of land covered by water, and household proximity to water [24,25]. An evaluation of U.S. NTM infections published in 2017 noted that, while MAC infections were common across the country, higher prevalence of MAB and *M. chelonae* infections was reported in the west [26]. Although it appears that there are distinct regional trends, it is unclear how geographic and environmental factors influence the prevalence of certain species and the risk of NTM infection.

NTM occurrence in source waters is also being investigated. Higher concentrations of *M. avium* in water have been associated with higher turbidity and particulate matter, possibly due to attachment to particles [27,28]. Furthermore, higher rates of NTM infection have been linked to the use of tap water derived from surface water sources versus from groundwater sources [29]. However, treatment and distribution system factors might have a greater impact than source water on NTM concentrations at the tap. For example, a source to tap monitoring study in Louisiana observed low NTM relative abundances in the Mississippi River water and drinking water leaving the treatment plant, but high relative abundances in distribution system and tap water [30]. At a minimum, this finding suggests that NTM persisted during distribution, but could mean that growth of NTM took place in the distribution system and building plumbing. Methods that allow monitoring of changes in

absolute abundances would be necessary to confirm NTM growth [31]. Although it appears that certain environmental conditions favor the proliferation of NTM in source water, these factors do not necessarily contribute to NTM concentrations at the tap. Further work is needed to assess the risks posed by the use of source waters containing NTM for drinking water production.

NTM Survival Through Drinking Water Treatment Processes

M. avium has been a potential concern in drinking water for decades and has been included on all U.S. Environmental Protection Agency Contaminant Candidate Lists. Nevertheless, NTM monitoring and reporting for drinking water is not required in the U.S. or other countries. This lack of surveillance has limited the evaluation of NTM removal through treatment systems. Much of the NTM-related drinking water research thus far has focused on NTM inactivation through disinfection. Pure cultures of *M. avium* require a disinfection dose, expressed as the product of disinfectant concentration and time of exposure (CT), up to 2,300-fold and 50-fold higher for 99.9 percent inactivation with chlorine and ozone, respectively, compared to *Escherichia coli* [32]. Similarly, inactivation of *M. fortuitum* and *Mycobacterium mucogenicum* with free chlorine requires a substantially higher CT than *E. coli* and *Bacillus subtilis* [33]. *M. avium* resistance to chloramine has also been observed, though reported values vary [32,34]. NTM inactivation at a given CT further varies based on the source of the strain (laboratory or environmentally-isolated strain), pH, nutrient availability, temperature, and whether the organism is sessile or planktonic [32,33,35,36]. Recently, increases in viable cell concentrations and the relative abundance of NTM were observed after initial exposure to ozone in a drinking water treatment plant's ozone contactors [37]. Further investigation determined that NTM were accumulating in biofilms and solids in the ozone contactors, which had formed due to non-ideal flow conditions. Such variations in inactivation for different growth conditions and strains make it difficult to simulate inactivation effectively in the laboratory and warrant further investigation in full-scale systems.

NTM survival has also been linked to entry into eukaryotic cells. Amoebae, such as *Hartmanella vermiformis* and *Acanthamoeba* spp., are commonly found in water systems and feed on bacteria via phagocytosis [38]. NTM possess the ability to survive phagocytosis and can live and sometimes replicate inside amoebae [39–41]. Surveys in drinking water have found that NTM and amoebae occurrence are often highly associated and that inactivation of *M. avium* inside *Acanthamoeba* required substantially higher CT values than free-living *M. avium* [42–44]. Furthermore, the demonstration that monochloramine exposure leads to the upregulation of mammalian cell entry gene 1 (*mce1*) in *M. avium*, which facilitates NTM entry into eukaryotic cells, is cause for concern (D Berry, PhD thesis, University of Michigan, 2009). This research suggests that, in addition to incidental amoebal uptake of NTM during grazing, some NTM might also initiate cell entry. The presence of amoebae in drinking water might therefore select for NTM that not only survive disinfection, but can also potentially infect other eukaryotic cells, including human cells. Given these findings, further investigation is needed to characterize how NTM respond to external pressures, such as oxidant (e.g., ozone, chlorine, chloramine) exposure, and to explore new methods for prevention of NTM infection.

NTM in Distribution Systems and Building Plumbing

Approximately 90% of the U.S. population receives drinking water treated in centralized treatment plants (U.S. Environmental Protection Agency; www.epa.gov/ground-water-and-drinking-water/safe-drinking-water-information-system-sdwis-federal-reporting). Treated water is transported through underground distribution systems and storage tanks, reaching consumers through building plumbing. Although utilities in the U.S. and many other countries provide a disinfectant residual to control microbial growth after treatment, dissipation of that residual, nutrient availability, and other factors result in drinking water that contains bacteria at levels as high as 10^6 to 10^8 cells/liter [45,46]. While NTM are resistant to both chlorine and chloramines, they are generally considered to be preferentially selected for in distribution systems where monochloramine is used as the residual disinfectant [11,47]. Other distribution system characteristics, such as water age, pipe material, accumulation of solids in storage tanks, and concentrations of dissolved metals could also influence NTM diversity, abundance, and growth [31,48,49]. Figure 2 summarizes interactions of chemical, biological, and physical factors that contribute to NTM occurrence in distribution systems. The complexity and heterogeneity of water systems make it challenging to link NTM growth and persistence to a few definable factors; more work is required to develop distribution system management strategies to mitigate the presence of NTM.

In contrast to distribution system studies, research on NTM in building plumbing is plentiful, likely due to the number of NTM infections linked to public buildings. Large surface area to volume ratios, intermittent stagnation, low disinfectant residuals, and warm temperatures make building plumbing a favorable environment for bacterial attachment and growth [50–53]. NTM have been found in numerous plumbing appurtenances, including faucets and showerheads [12,22,54]. An analysis of the microbial composition of showerhead biofilms in U.S. cities found significant enrichment for MAC spp., with higher relative abundances of MAC spp. in biofilms compared to in the water feeding into showerheads [54]. Higher relative abundances of NTM have also been observed in showerheads fed by municipal water versus well water [12,54]. A recent survey of homes in Ann Arbor, Michigan receiving chloraminated water found NTM in all building plumbing water and biomass samples collected [31]. NTM abundance has been linked to various abiotic and biotic parameters in building plumbing, including iron concentration, water age, disinfectant type, and the presence of amoebae [11,31,42]. However, further research is needed to better characterize the concentrations of clinically relevant strains and understand how these concentrations might correlate to specific chemical and physical parameters.

Potential interventions for OP control in building plumbing include chemical and physical means of deselection. Studies evaluating on-site disinfectant addition have shown varying levels of efficacy and note the potential for corrosion and scaling caused by the added oxidants [55,56]. One study also noted the increase in relative abundance of NTM with the implementation of on-site monochloramine disinfection [57]. An evaluation of UV irradiation found that the dose required for inactivation of 15 strains of *M. avium* subsp. *hominissuis* varied widely across strains and was substantially higher than doses typically used in water treatment [58]. While such studies provide insights into potential control

strategies for NTM, more work is needed to identify mitigation strategies that do not result in unintended consequences (e.g., selection for other OPs).

Routes of Exposure and Infection

Studies have repeatedly shown that rates of NTM pulmonary infection around the world are increasing. While the reasons for this rise are unclear, increased exposure to NTM might be a contributing factor [59]. Linking NTM infections to a particular source is challenging and is complicated by often long periods of time between NTM exposure and diagnosis, lack of standardized methods for strain genotyping, and the pervasiveness of NTM in the environment. The most probable routes of NTM pulmonary infection are through inhalation and aspiration, whereas contact with contaminated surfaces is likely significant for skin, soft tissue, and post-operative infections [4,16,60] (Figure 3). While NTM are believed to be inhaled primarily through water aerosols, complex air flow dynamics and low biomass yields make nucleic acid-based analyses of aerosols challenging. Despite these challenges, measurable concentrations of NTM in aerosols have been detected in air samples from bathrooms, showers, humidifiers, hot tubs, and therapy pools [61–64]. Further studies on NTM quantification and detection in aerosol samples are required to elucidate the link between water and aerosolized NTM.

While NTM can infect humans through multiple routes, the risk of infection is highly dependent on the host. Risk factors that contribute to a person's susceptibility include increased age and pre-existing health conditions. Persons with lung pathology or compromised immune systems, such as those with chronic obstructive pulmonary disease, cystic fibrosis, and human immunodeficiency virus infection, are at particular risk of pulmonary NTM infection [65–68]. Genetic factors and use of some immunosuppressing medications are also thought to increase susceptibility [69–71]. Although efforts have been made to assess the risk posed by NTM in water through quantitative microbial risk assessment, so far these studies do not capture the full breadth of the complexity associated with NTM in drinking water [72–74]. This type of risk assessment is complex, and drinking water-specific studies are needed to elucidate how host and environmental factors influence risk of NTM infection.

Future Work

Although our ability to detect and identify NTM has greatly improved over the last two decades, little is known about how the observed concentrations of various NTM in drinking water correlate to the disease burden. As shown in Figure 3, considerable work is needed to assess how various exposure mechanisms, concentrations of NTM, and host-specific factors contribute to risk of pulmonary infection. Aerosolization of NTM is in particular need of additional investigation given the hypothesis that aerosols are a central route of exposure. Recent studies with other infectious bacteria have shown that certain species and strains were preferentially aerosolized, and that ease of aerosolization might correlate with strains more frequently associated with infection [75,76]. Similar studies are needed to determine how NTM concentrations in water correlate with levels in aerosols to narrow the focus of risk assessment to strains that are most readily aerosolized. Compared to other, better-

studied bacteria, relatively little is known about how exposure to NTM correlates with risk of infection and what can be considered an infective dose. The risk of NTM drug resistance is also of growing interest, as NTM infections typically require extended treatment with multiple antimicrobials and recurrence of infection is common [77,78]. Given the ubiquity of NTM in water and other environments, complete elimination or inactivation of viable organisms during drinking water treatment and distribution might not be possible. Rather, NTM levels in drinking water must be managed to minimize the risk of infection, as is done with other microorganisms of concern.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Nontuberculous mycobacteria (NTM) are pervasive in drinking water systems.
- NTM are resistant to disinfection and are found in pipe and showerhead biofilms.
- Inhalation and/or aspiration of drinking water might cause NTM pulmonary infections.
- Improved infection reporting is needed to better characterize NTM health burden.
- Linking NTM infections to water sources is difficult and requires further study.

Figure 1. Dendrogram showing relatedness of NTM species and strains based on *rpoB* gene sequences. Text colors refer to the types of samples the sequences were obtained from (light blue - home drinking water samples; green – drinking water treatment plant samples; dark blue – sequences found in both home and drinking water samples; orange – water from hospital equipment; yellow – hospital drinking water; red – clinical respiratory samples; black – whole genome sequences at least 85% complete added to NCBI prior to 2015). Sources provided in SI.

Figure 2.
Factors influencing NTM concentrations in distribution systems

Figure 3. Overview of possible NTM infection and exposure routes through drinking water and suggested future work needed for NTM infectivity and pulmonary infection prevention.

Table 1

Common methods for the identification and quantification of NTM

Type of analysis/target molecule	Method	Target	Typical level of identification	Type of sample that can be processed	Citation
Culture-based	Lowenstein-Jensen slant	Viable and culturable <i>Mycobacterium</i> cells	Presumptive genus-level - although subsequent molecular or biochemical methods are required to confirm	Mixed culture sample with subsequent passage to isolate pure cultures	[11,12,23,28,32-36,3941,44,58,61-63,78-80]
	Middlebrook medium				
	PCR	16S rRNA gene	genus-level - presence or absence	Pure culture or mixed culture sample	[81]
		16S - 23S rRNA gene ITS region	species-level - presence or absence	Pure culture or mixed culture sample	[82]
Nucleic acids		Random amplified polymorphic DNA (RAPD)	Species-, subspecies-level	Pure culture	[83]
	qPCR	16S rRNA gene	genus-level	Mixed culture sample	[27,49,53,54]
		<i>atpE</i> gene	genus-level	Mixed culture sample	[31,40,42]
		16S - 23S rRNA gene ITS region	species-level	Mixed culture sample	[43,48,52]
		<i>hsp65</i> gene	genus- or species-level depending on primers	Mixed culture sample	[84]
		Whole genome	sub-species level ¹	Pure culture or mixed culture sample	[15,23,61,79]
	Sequencing	16S rRNA gene	genus-, complex-, or species-level ²	Pure culture or mixed culture sample	[11,12,19,30,37,4750,53,54,57,62,85,86]
		<i>rpoB</i> gene	genus-, complex-, or species-level ²	Pure culture or mixed culture sample	[20,22,39,42]
		<i>hsp65</i> gene	genus-, complex-, or species-level ²	Pure culture or mixed culture sample	[11,12,86]
		16S - 23S rRNA gene ITS region	genus-, complex-, or species-level ²	Pure culture or mixed culture sample	[54]
		Multi-locus sequence typing (MLST)	species-level	Pure culture	[17,86]
	Pulsed-field gel electrophoresis	Viable and culturable <i>Mycobacterium</i> cells	Complex- or species-level	Pure culture	[5,78,79]
	PCR-restriction fragment length polymorphism (PCR-RFLP)	<i>rpoB</i> gene	sub-species level	Pure culture	[87]

Type of analysis/target molecule	Method	Target	Typical level of identification	Type of sample that can be processed	Citation
	Microarray and probe hybridization	<i>hsp65</i> gene	species-level	Pure culture	[23,28,88]
		16S - 23S rRNA gene ITS region	complex- or species level	Pure culture	[89]
		16S rRNA gene	complex- or species level	Mixed culture sample	[81,85]
	High performance liquid chromatography (HPLC)	<i>gyrB</i> gene	Complex or species level	Mixed culture sample	[90]
Lipids	Matrix-assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF MS)	Mycolic acids	Species-level	Pure culture	[79,91]
Proteins		Cellular proteins	Complex or species level	Pure culture	[79,92]

¹level of identification and genome completeness achieved vary based on sequencing platform and level of diversity in mixed samples,

²level of identification achieved depends on the target gene, length of amplicon, and sequencing platform used.