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REVIEW ARTICLE

Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment

J.O. Falkinham, III

Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

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Correspondence
Joseph O. Falkinham, III, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061-0406, USA. E-mail: jofiii@vt.edu

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Summary

A majority of the *Mycobacterium* species, called the nontuberculous mycobacteria (NTM), are natural inhabitants of natural waters, engineered water systems, and soils. As a consequence of their ubiquitous distribution, humans are surrounded by these opportunistic pathogens. A cardinal feature of mycobacterial cells is the presence of a hydrophobic, lipid-rich outer membrane. The hydrophobicity of NTM is a major determinant of aerosolization, surface adherence, biofilm-formation, and disinfectant- and antibiotic resistance. The NTM are oligotrophs, able to grow at low carbon levels [>50 μg assimilable organic carbon (AOC) l^{-1}], making them effective competitors in low nutrient, and disinfected environments (drinking water). Biofilm formation and oligotrophy lead to survival, persistence, and growth in drinking water distribution systems. In addition to their role as human and animal pathogens, the widespread distribution of NTM in the environment, coupled with their ability to degrade and metabolize a variety of complex hydrocarbons including pollutants, suggests that NTM may be agents of nutrient cycling.

Introduction

For almost 30 years, based on the absence of person-to-person transmission, it has been widely accepted that the source of nontuberculous mycobacteria (NTM) infecting humans is the environment (Wolinsky 1979; Marras and Daley 2002). These mycobacteria constitute the majority of species in the genus *Mycobacterium* (Tortoli 2003) and are important environmental opportunistic pathogens of humans, animals, poultry, and fish (Falkinham 1996, 2002; Wayne and Sramek 1992; Biet *et al.* 2005). Evidence collected over the past 30 years has documented that the NTM are normal inhabitants of a variety of environmental habitats that are shared with humans and animals, including natural waters, drinking water distribution systems, and soils (Falkinham 1996, 2002). The fact that NTM occupy habitats shared with humans and animals is clearly a major determinant in the acquisition of disease. NTM appear in high numbers in waters and biofilms in drinking water distribution systems (Covert *et al.* 1999; Falkinham *et al.* 2001) where they can enter household plumbing (Falkinham *et al.* 2008). In this brief review, the habitats, transmission routes, and physiological determinants of environmental occupancy by NTM will be identified.

The major determinant of NTM ecology and epidemiology is the presence of a lipid-rich outer membrane (Nikaido *et al.* 1993; Brennan and Nikaido 1995; Daffe and Draper 1998; Hoffman *et al.* 2008). The outer membrane's long chain mycolic acids contribute to the hydrophobicity, impermeability, and slow growth of both slowly and rapidly growing mycobacteria (Brennan and Nikaido 1995). Those features, in turn, lead to the preferential attachment to surfaces (Bendinger *et al.* 1993) and resistance to disinfectants and antibiotics (Rastogi *et al.* 1981; Jarlier and Nikaido 1994). NTM are oligotrophs (George *et al.* 1980; Hall-Stoodley *et al.* 1999; Norton *et al.* 2004) and able to grow on a variety of organic compounds (Goodfellow and Magee 1998) including some found in water and soil (e.g. humic and fulvic acids; Kirschner *et al.* 1999). Investigations of the physiological traits of NTM and the chemical characteristics of waters

and soils correlated with NTM presence have led to identification of NTM characteristics that are determinants of their widespread distribution and presence in some unusual habitats (e.g. acid, brown water swamps, boreal and peat rich soils, and metal working fluids). The study of the physiological ecology of NTM has led to insight into habitats that they might possibly occupy (e.g. polluted soils) and the suggestion that NTM may be agents of nutrient cycling.

Environmental opportunistic mycobacteria

The most common NTM isolated from humans are listed in Table 1. All of the species listed in Table 1 as well as most other *Mycobacterium* spp. are opportunistic pathogens. In addition to their isolation from patients suffering from mycobacterial disease, most have been recovered from environmental habitats. NTM disease has been described and its risk factors have been identified (Wolinsky 1979; O'Brien *et al.* 2000; Marras and Daley 2002; Field *et al.* 2004; Fowler *et al.* 2006). These include: (i) reduced immune competence as a result of human immunodeficiency virus (HIV) infection, cancer, chemotherapy, or immunosuppression associated with transplantation; (ii) pre-existing lung disease, including chronic obstructive pulmonary disease (COPD), pneumoconiosis and silicosis, and prior tuberculosis; (iii) altered chest architecture; (iv) alcoholism, and (v) smoking. Recently, two additional risk factors, mutations in either the cystic fibrosis transmembrane conductance regulator (CFTR) or the α-1-antitrypsin gene have been associated with pulmonary disease caused by both slowly and rapidly growing NTM (Kim *et al.* 2005; Rodman *et al.* 2005; Chan *et al.* 2007). Evidence that persons heterozygous for CFTR mutations (Kim *et al.* 2005) coupled with the fact that the frequency of CFTR heterozygotes is 0·04 (1 in 25), suggests that a substantial proportion of humans are at risk for NTM disease. The incidence of NTM lung disease is increasing (Marras *et al.* 2007), particularly among the elderly slender individuals (Prince *et al.* 1989; Reich and Johnson 1991; Kennedy and Weber 1994). As use of immunosuppressive agents and the proportion of elderly are expected to increase and the survival of risk groups (e.g. cystic fibrosis patients) is expected to lengthen in the developed world, it follows that the incidence of NTM disease will continue to increase. Because antimycobacterial therapy requires a cocktail of multiple drugs over prolonged periods of time and carries with it the possibility of drug side-effects (Griffith *et al.* 2007) and humans are surrounded by mycobacteria (see following), research ought to be directed towards identifying host and behavioural factors leading to enhanced susceptibility to NTM infection and devising means to reduce exposure.

Table 1 Environmental opportunistic mycobacteria

Species	Reference
Slowly growing mycobacteria (colony formation ≥ 7 days)	
Mycobacterium avium	Prince *et al.* 1989
Mycobacterium intracellulare	Prince *et al.* 1989
Mycobacterium kansasii	Lillo *et al.* 1990; Alcaide *et al.* 1997
Mycobacterium xenopi	Costrini *et al.* 1981
Mycobacterium marinum	Aubry *et al.* 2002
Mycobacterium malmoense	Zaugg *et al.* 1993
Mycobacterium simiae	Conger *et al.* 2004
Rapidly growing mycobacteria (colony formation 3–7 days)	
Mycobacterium abscessus	Wallace 1994; Wallace *et al.* 1998; Jonsson *et al.* 2007
Mycobacterium chelonae	Wallace 1994; Wallace *et al.* 1998; Uslan *et al.* 2006
Mycobacterium fortuitum	Wallace 1994; Wallace *et al.* 1998

Habitats of environmental mycobacteria

Habitats from which environmental opportunistic mycobacteria have been isolated are listed in Table 2. The most important in terms of human and animal health are engineered habitats, particularly drinking water distribution systems, hospital water systems, and household plumbing. Here, human and mycobacterial habitats overlap permitting recurrent exposure. The original focus of environmental surveys was on natural waters and soils (Falkinham *et al.* 1980; Brooks *et al.* 1984), driven by evidence of higher skin test sensitivity in individuals in the southeastern United States (Edwards *et al.* 1969). However, the outbreak of the AIDS epidemic starting in 1982 and reports of *Mycobacterium avium* infections in AIDS patients across the United States (Greene *et al.* 1982) alerted mycobacteriologists to the fact that *M. avium* and other NTM were likely widely distributed across the United States. Recovery of *M. avium* and other NTM from drinking water (Du Moulin and Stottmeier 1986; Fischeder *et al.* 1991; von Reyn *et al.* 1993) led to thorough studies of NTM in United States drinking water distribution systems (Covert *et al.* 1999; Glover *et al.* 1994; Falkinham *et al.* 2001) and proof that drinking water distribution systems (von Reyn *et al.* 1994), hospitals (Wallace *et al.* 1998), and household plumbing (Falkinham *et al.* 2008) are sources of human infection. In addition to water, soil is a habitat for NTM (Brooks *et al.* 1984; Iivanainen *et al.* 1997) and exposure to soil has been shown to be a risk factor for acquisition of *M. avium* disease (Reed *et al.* 2006; De Groote *et al.* 2006).

Table 2 Habitats of environmental opportunistic mycobacteria

Habitat	Reference
Natural waters	Falkinham *et al.* 1980; von Reyn *et al.* 1993
Drinking water distribution systems	Covert *et al.* 1999; Falkinham *et al.* 2001
Biofilms in drinking water distribution systems	Falkinham *et al.* 2001; Torvinen *et al.* 2004
Building, hospital, and household plumbing	Du Moulin *et al.* 1988; Wallace *et al.* 1998; Nishiuchi *et al.* 2007; Falkinham *et al.* 2008
Hot tubs and spas	Embil *et al.* 1997; Kahana *et al.* 1997; Mangione *et al.* 2001; Marras *et al.* 2005
Natural and household/building aerosols	Falkinham *et al.* 2008
Boreal forest soils and peats	Iivanainen *et al.* 1997, 1999
Acidic, brown-water swamps	Kirschner *et al.* 1992
Potting soils	De Groote *et al.* 2006
Metal removal fluid systems	Bernstein *et al.* 1995; Shelton *et al.* 1999; Moore *et al.* 2000

Factors influencing distribution of mycobacteria in the environment

Physiological determinants

The characteristics of NTM that influence their presence and distribution in the environment are listed in Table 3. It is likely that the same factors are determinants for both slowly growing and rapidly growing NTM. The rapidly growing NTM, still grow substantially slower (i.e. 0·2–0·5 days per generation) than many other bacteria Because of their slow growth and impermeability, one would wonder why NTM are so widely distributed; they should be poor competitors. That is likely the case in nutrient-rich habitats, but both slowly (George *et al.* 1980) and rapidly growing NTM (Hall-Stoodley *et al.* 1999) thrive in marginal environments where their traits permit their survival and proliferation. In a number of instances (i.e. drinking water distribution systems), human intervention (e.g. disinfection) contributes to selection for proliferation and persistence of NTM.

The major structural feature of NTM is the lipid-rich outer membrane (Brennan and Nikaido 1995; Daffe and Draper 1998). The outer membrane is a true membrane, containing porins, and rich in unique mycolic acids (Hoffman *et al.* 2008). Two factors associated with the outer membrane limit growth rates for the slowly growing NTM and to a lesser extent the rapidly growing NTM; the cost of synthesis of the long-chain mycolic acids and impermeability to hydrophilic nutrients. However, those costs are more than offset by the presence of a hydrophobic barrier that promotes the attachment of NTM to surfaces (Bendinger *et al.* 1993) permitting their persistence in habitats where they could be washed out. That hydrophobic barrier also protects both slowly (e.g. *M. avium*) and rapidly (e.g. *Mycobacterium chelonae*) growing NTM cells from a wide range of antimicrobial agents (Rastogi *et al.* 1981; Jarlier and Nikaido 1990). The high intrinsic resistance to antibiotics makes it difficult to identify resistance mechanisms in slowly growing NTM based on efflux or drug-inactivating enzymes, although both types of resistance mechanisms have been identified in rapidly growing NTM (Webb and Davies 1998).

Table 3 Factors influencing distribution of mycobacteria in natural and human engineered environments

	Impacts in habitats	
Factor	Natural habitats	Engineered habitats
Hydrophobicity	Attach to particulates Biofilm formation Concentration at air : water interfaces Hydrocarbon utilization	Attach to surfaces Biofilm formation Antimicrobial resistance Hydrocarbon utilization
Growth at low pH	High numbers in acidic, brown water swamps and boreal (peat) soils	
Humic and fulvic acid growth stimulation	High numbers in acidic, brown water swamps and boreal (peat) soils	Growth in drinking water distribution systems and household plumbing
Temperature resistance	Survive in hot springs	Survive in buildings and home hot water systems

A second characteristic limiting NTM growth is the low number (i.e. one or two copies) of rRNA operons (Bercovier *et al.* 1986; Cox 2004; Cox and Cook 2007). In addition to limiting the rate of protein synthesis, rRNA synthesis is a major determinant of growth (Maaløe and Kjeldgaard 1966; Cox 2004; Cox and Cook 2007). Recent studies of the relationship between rRNA operon copy number and growth in *Escherichia coli* has suggested that low copy number restricts the ability of bacteria to respond to up shifts in nutrient ability (Condon *et al.* 1995). However, there is a benefit to slow growth rate. Highest rates of bacterial killing are experienced by rapidly growing cells, in part due to the fact that antimicrobial agents kill by selective inhibition of cellular processes, such as cell wall, DNA, RNA, and protein synthesis. Inhibition of one process, while others continue leads to unbalanced growth and death (Maaløe and Kjeldgaard 1966). In the presence of antimicrobial agents, rapidly growing cells reach unbalanced growth, leading to death (Maaløe and Kjeldgaard 1966). In contrast to rapidly growing bacteria, imbalance in both slowly and rapidly growing NTM can be counteracted because there is adequate time for their adaptation to changed conditions before the onset of a lethal event. Mycobacterial cells have metabolic rates that are the same as other bacteria; they are not slow metabolizers. Examples of NTM adaptation include the onset of a dormant state to cells exposed to anaerobiosis (e.g. *Mycobacterium smegmatis*; Dick *et al.* 1998) or starvation (e.g. *M. avium*; Archuleta *et al.* 2005) and the increased resistance of both rapidly and slowly growing NTM in biofilms to antimicrobial agents (Bardouniotis *et al.* 2001; Steed and Falkinham 2006; Falkinham 2007).

Genetic variation in mycobacteria

Genetic variation in NTM species is manifest in virulence differences, colony morphology variation, appearance of different geographic types, and clonal variation in environmental habitats and in patients. The most troubling aspect of genetic variation in NTM is the wide differences in virulence of isolates of the same species and the lack of tests for assessing virulence; particularly for *M. avium* and *Mycobacterium intracellulare* (McGarvey and Bermudez 2002). Unfortunately animal or macrophage growth measurements cannot be used reasonably for screening environmental isolates for potential virulence. Knowledge of virulence markers is essential for a risk analysis of NTM in drinking water. One study did demonstrate that plasmid-carrying strains of *M. avium* were likely more virulent in the beige mouse compared with plasmid-free, but unfortunately the strains were not isogenic (Reddy *et al.* 1994). Such a result begs further investigation. There is yet no described genetic or phenotypic marker whose appearance correlates with virulence.

Colony variation is the most thoroughly investigated phenomenon, although its genetic basis is in NTM still undescribed; even in *M. avium* where the majority of work has been performed. *Mycobacterium avium* colonies switch between two types, smooth D (opaque) and smooth T (transparent) (McCarthy 1970; Woodley and David 1976; Cangelosi *et al.* 2001). Similar colony variant switching has also been reported for *Mycobacterium abscessus* (Howard *et al.* 2006). These differences are significant because the transparent types are relatively more virulent, hydrophobic, and antibiotic-resistant compared with the hydrophilic, less virulent, and more antibiotic-sensitive opaque types (McCarthy 1970; Schaefer *et al.* 1970; Kajioka and Hui 1978). The rate of switching (i.e. opaque to transparent and transparent to opaque) occur at frequencies of 1 per 1000 colonies (McCarthy 1970; Woodley and David 1976; Cangelosi *et al.* 2001). The high frequency of the alternative colony types has led to the proposal that switching was influenced by loss of plasmid DNA in *M. intracellulare* (Mizuguchi *et al.* 1981). NTM colonies on agar medium, cells in biofilms, and patients will contain cells of both colony types. Transparent types are isolated from patients, rather than opaque types (Schaefer *et al.* 1970). Laboratory cultivation appears to select for the hydrophilic, faster-growing opaque types (Kajioka and Hui 1978; Howard *et al.* 2006); an observation suggesting that mycobacteriologists take special care to ensure their cultures are transparent types, better able to represent the bacteria in patients.

Among patient and environmental isolates of single mycobacterial species, e.g. *M. avium* or *M. intracellulare*, there is a great deal of genetic variation exemplified by different pulsed field gel electrophoresis (PFGE; Mazurek *et al.* 1993), insertion sequence restriction fragment length polymorphism (IS-RFLP; van Soolingen *et al.* 1998), and repetitive sequence polymorphisms (rep-PCR; Cangelosi *et al.* 2004). Calculations of indices of discrimination for all three methods of DNA fingerprinting (Hunter and Gaston 1988) show that they provide the discrimination for source tracking. The breadth of different types for each method, means that finding a match, as for example between an environmental and patient isolate, is highly significant and can be used to identify sources of infection by the environmental opportunistic pathogenic mycobacteria. Clonal variation can be observed in isolates recovered from a single household's plumbing as well as from individual patients. Multilocus sequencing (MLS), namely determining the sequence of selected genes, has also been used for taxonomic studies of members of the *M. avium* complex (*M. avium, M. intracellulare*, and *M. avium* subsp. *paratuberculosis*; Turenne *et al.* 2008).

Here, it should be pointed out, that MLS typing may not necessarily be useful for linking patient and environmental isolates, but is useful for establishing taxonomic groupings and identifying geographical types.

Phenotypic variation in environmental mycobacteria

Intracellular growth of *M. avium* strains in either macrophages or amoebae results in increased virulence (Cirillo *et al.* 1997) and antibiotic resistance (Miltner and Bermudez 2000). Because a substantial proportion of mycobacterial cells in infected patients and animals are growing in macrophages or other phagocytic cells, measurement of virulence of mycobacterial strains grown in the laboratory medium, may inaccurately underestimate virulence.

Growth of *M. avium* in biofilms on glass, copper, galvanized steel, or PVC results in cells that are transiently more resistant to antimicrobial agents (Steed and Falkinham 2006) and antibiotics (Falkinham *et al.* 2008). This increased resistance is not because of difficulties in penetration of antimicrobial agents in the layers of cells and extracellular matrix, but is displayed by cells recovered from biofilms, washed, and exposed to antimicrobial agents in suspension (Steed and Falkinham 2006; Falkinham *et al.* 2008). The increased resistance is adaptive, not genetic, because resistance is lost after 1 day's growth of biofilm-grown cells in suspension (Steed and Falkinham 2006). These observations point to the fact that although NTM are slow growing, their metabolism is as rapid as other bacteria and they are capable, thereby, of rapid adaptation (e.g. the synthesis of new proteins) to enhance survival.

Mycobacteria in drinking water systems and household plumbing

Cell surface hydrophobicity is a major determinant of the presence of NTM in drinking water distribution systems and household plumbing. Both rapidly and slowly growing NTM colonize drinking water systems via their attachment to particulates that enter the treatment plant and to the formation of biofilms in the distribution system (Schulze-Röbbecke and Fischeder 1989; Schulze-Röbbecke *et al.* 1992; Wallace *et al.* 1998; Falkinham *et al.* 2001; Torvinen *et al.* 2004). Hydrophobicity-driven surface attachment (Bendinger *et al.* 1993) prevents flushing from the system as NTM growth rates cannot keep pace with dilution owing to water flow. Further, slow growth and disinfectant resistance in *M. avium* have been shown to contribute to survival, growth, and persistence (Taylor *et al.* 2000; Falkinham 2003). Disinfection kills off competitors, consequently selecting for the oligotrophic NTM that can grow on the low levels of nutrient (George *et al.* 1980; Hall-Stoodley *et al.* 1999; Norton *et al.* 2004). Biofilm formation results in increased disinfectant resistance of *M. avium* and *M. intracellulare* (Steed and Falkinham 2006) and *Mycobacterium phlei* cells (Bardouniotis *et al.* 2001). All those factors likely contributed to the increase in *M. avium* numbers in drinking water distribution systems, the further the distance from the treatment plant (Falkinham *et al.* 2001).

Household and building plumbing systems yield NTM. *Mycobacterium avium* numbers in hospital hot water systems were increased relative to the incoming water (Du Moulin *et al.* 1988) and bathroom plumbing of patients with *M. avium* complex (MAC) infections yielded MAC (Nishiuchi *et al.* 2007). In a study of household plumbing where an individual with *M. avium* pulmonary infection resided, *M. avium* isolates that were clonally related to the patient isolate were recovered; including one from a bathroom shower head (Falkinham *et al.* 2008). The same factors affecting NTM numbers and distribution in a drinking water distribution system likely operate in household plumbing. In most instances, there is a low residual disinfectant level in household waters. Further, it is likely that both rapidly and slowly growing NTM can survive in hot water heaters and hot water pipes because they survive temperatures of between 50 and 55°C (Schulze-Röbbecke and Bucholtz 1992; Santos *et al.* 2007). Unless hot water heater temperatures are maintained above 50°C, NTM may proliferate in household hot waters. Low oxygen concentrations as a consequence of reduced or intermittent water flow in households and microbial-driven oxygen consumption may not limit NTM growth. *Mycobacterium smegmatis* can adapt to survive at low oxygen concentrations (Dick *et al.* 1998) and strains of *M. avium* and *M. intracellulare* can grow at 6–12% oxygen (Lewis and Falkinham, unpublished). Thus, household plumbing provides a stable, nutrient-limited, disinfectant-containing habitat that is ideal for NTM growth and persistence.

Mycobacteria in metal removal fluids

Recently, it has been proposed that hypersensitivity pneumonitis (HP) in automobile workers is owing to the presence of NTM in metal removal fluid (MRF) aerosols (Bernstein *et al.* 1995; Shelton *et al.* 1999). The association between mycobacterial exposure and HP is not new. HP has been shown to follow exposure to mycobacterial cells (Huttunen *et al.* 2000; Marras *et al.* 2005) or mycobacterial cell constituents (Richerson *et al.* 1982). MRF are water : organic emulsions designed to cool metal-cutting and -grinding tools and carry off small fragments of metal. Metal-working fluids are diluted in water to form emulsions and it is likely that the source of the

NTM in MRF is the water. A rapidly growing NTM species, *Mycobacterium immunogenum*, can attach to pipe and conduit surfaces and can grow in used, but not fresh, MRF (Moore *et al.* 2000). Their growth in MRF systems is likely because of their hydrophobicity-driven attachment to surfaces (Bendinger *et al.* 1993; Stelmack *et al.* 1999) and metabolism of some MRF hydrocarbon constituents (Krulwich and Pelliccione 1979). Other micro-organisms can grow in MRF (Buers *et al.* 1997) and their growth leads to reduced efficacy of MRF in removing particulates (Steinhauer and Goroncy-Bermes 2007). To prevent and reduce microbial numbers, disinfectants can be added to MRF. As appears to be the case in drinking water distribution systems, disinfection of MRF leads to killing of most micro-organisms and leaves the disinfectant-resistant NTM to proliferate in the absence of competition.

Mycobacteria in polluted environments

Evidence of the presence of rapidly growing NTM in polluted soils (Wang *et al.* 2006), suggests that rapidly growing NTM may be important agents of mineralization of pollutants. Before that report, no one considered rapidly growing NTM as agents for pollutant degradation or remediation. Further, because of their slow growth, it is unlikely that even rapidly growing NTM colonies would have been detected using conventional microbiological practice. Polluted sites may be ideal habitats for rapidly growing NTM because they (i) can metabolize a variety of major groundwater hydrocarbon pollutants (e.g. Burback and Perry 1993; Heitkamp *et al.* 1988), (ii) attach to particulates where pollutants are concentrated (Stelmack *et al.* 1999), (iii) they are resistant to antimicrobial agents (Bardouniotis *et al.* 2001), (iv) hydrophobicity-driven adherence to particulates would prevent flushing from polluted sites (Bendinger *et al.* 1993), and (v) limited oxygen levels are unlikely to restrict mycobacterial metabolism (Dick *et al.* 1998). Based on that analysis of polluted sites, it is possible that rapidly growing NTM may be agents of pollutant metabolism and mineralization.

Transmission of mycobacteria

Aerosol droplet transmission

Slowly growing NTM are readily transmitted between habitats. Cells of *M. avium* and *M. intracellulare* are readily aerosolized from water to air, via droplet formation (Parker *et al.* 1983). Hydrophobic NTM cells adhere to air bubbles rising in a water column and, at the water surface, the bubble bursts ejecting droplets to heights of 10 cm (Parker *et al.* 1983). *Mycobacterium avium* and *M. intracellulare* densities are 1000 to 10 000-fold higher in the ejected jet droplets than in the water (Parker *et al.* 1983) and are small enough (<100 μm diameter) to be readily carried off (Wendt *et al.* 1980). A substantial proportion of the droplets are small enough (≤5 μm diameter) to enter human alveoli (Wendt *et al.* 1980; Parker *et al.* 1983). Evidence of *M. avium* pulmonary infection and disease associated with exposure to aerosols generated in showers (Falkinham *et al.* 2008) and hot tubs and spas (Embil *et al.* 1997; Kahana *et al.* 1997; Mangione *et al.* 2001) is indirect evidence of aerosol-borne mycobacterial infection. In addition, HP has been associated with *M. avium* exposure associated with a hot tub (Rickman *et al.* 2002).

Waterborne transmission

Waters and soils are possible sources of *M. avium* cervical lymphadenitis in children (Wolinsky 1995). The peak age of children with cervical lymphadenitis (i.e. 6 months to 2 years) suggests occurrence at the time of childhood exploration of their environment and the emergence of teeth. Soil and water both contain *M. avium* and trauma to gums associated with erupting teeth provides the source and route of infection, respectively. Recently, gastric oesophageal reflux disease (GERD) has been suggested as a possible mediator of NTM pulmonary disease (Thomson *et al.* 2007; Koh *et al.* 2007). Here, the scenario involves swallowing NTM and gastric reflux leading to aspiration into the lungs. The acid resistance of *M. avium* (Bodmer *et al.* 2000) and other NTM (Portaels and Pattyn 1982) to pH levels encountered in the stomach is consistent with this possible route of infection. Animals, as well, are subject to NTM infection via water (Biet *et al.* 2005).

Transmission in soils and dusts

Numbers of rapidly and slowly growing NTM are relatively high in soils, particularly acidic, boreal forest soils and peats (Iivanainen *et al.* 1997), water draining from peat-rich soils (Iivanainen *et al.* 1999), and acidic, brown water swamps of the eastern coastal United States (Kirschner *et al.* 1992). High NTM numbers, particularly *M. avium* and *M. intracellulare* are found in commercially available, peat-rich potting soil (Yajko *et al.* 1995; De Groote *et al.* 2006). Hydrophobicity drives the adherence of NTM to soil particles (Bendinger *et al.* 1993; Stelmack *et al.* 1999) and thus, they can be readily aerosolized as dusts produced from dry soils. In fact, NTM of the same species and sharing the same DNA fingerprint with those of the patient were recovered from dusts generated by dropping the patient's own soil (De Groote et al. 2006).

Reducing mycobacterial numbers

Risks of disinfection

Because drug therapy of infection and disease caused by NTM often requires multiple antibiotics for prolonged periods with their attendant side-effects (Griffith *et al.* 2007), it is reasonable to conceive and investigate methods for reducing NTM exposure. At the outset, I suggest that disinfection should be avoided. Disinfection likely kills off most micro-organisms except NTM, selecting and creating a habitat with few NTM competitors. It was suggested that the substantial reduction of the normal microbial flora of the lungs with antibiotic therapy for bronchitis in a single patient, likely contributed to *M. avium* pulmonary disease linked to exposure in that patient's shower (Falkinham *et al.* 2008). Clearly that situation may be rare, but disinfection of engineered habitats (e.g. drinking water systems) may actually enrich the population for NTM. Effective chlorine disinfection for *M. avium* and *M. intracellulare* requires exposures of greater than 1 mg l^{-1} for longer than 2 hours (Taylor *et al.* 2000).

Filtration

Water filtration has been shown to reduce NTM numbers, but without changing the filter regularly (<3 weeks), the filter can become a source (Rodgers *et al.* 1999). Filters provide an ideal habitat for NTM; they attach and can grow on the filter material on the organic compounds collected and concentrated on the filters, even if the filter is impregnated with an antimicrobial agent (e.g. silver; Rodgers *et al.* 1999). NTM numbers in drinking water distribution systems are higher in systems with higher turbidity (Falkinham *et al.* 2001), likely because of the hydrophobicity-driven adherence of NTM to soil particulates (Bendinger *et al.* 1993; Stelmack *et al.* 1999). Thus, reduction of water turbidity would be expected to reduce NTM numbers in both water treatment systems and households.

Ultraviolet irradiation

A review of the literature documents that members of the genus *Mycobacterium* are as susceptible to short-wave length ultraviolet irradiation (UVC) as is *E. coli* (Collins 1971; David *et al.* 1971; David 1973; McCarthy and Schaefer 1974). Further, there is evidence that UV irradiation was effective in reducing numbers of mycobacteria in aquarium tanks (Agbalika *et al.* 1984). This suggests that UV irradiation may be a viable method for reducing numbers of water-borne NTM in drinking water distribution systems, buildings, and household plumbing. However, as noted for disinfection, this approach may have a drawback that requires evaluation. UV irradiation is mutagenic and it will be important to determine whether UV disinfection leads to an increase in mutants among the survivors.

Copper-silver ions

Copper-silver ion-generating systems have been installed in hospitals and public buildings to successfully reduce numbers of *Legionella* (Liu *et al.* 1998). Copper-silver ions also kill *M. avium* (Lin *et al.* 1998) and other NTM, albeit requiring higher dosages compared with *Legionella* (Kusnetsov *et al.* 2001). Because of the proven efficacy of copper-silver ion-generating systems in reducing *Legionella* numbers in a substantial number of hospitals and public buildings, it will be of value to determine their utility in reducing NTM numbers as well.

Exploiting mycobacterial hydrophobicity

Finally, it is possible that the hydrophobicity of NTM has not been exploited as a target for selective removal. NTM are the most hydrophobic of the micro-organisms (van Oss *et al.* 1975). NTM cells can be almost entirely removed (>99·9%) from aqueous suspensions by partitioning in an organic solvent such as hexadecane (Stormer and Falkinham 1989). Filters coated with hydrophobic materials (e.g. paraffin) could be used to selectively remove NTM from waters, aerosols, or dusts. NTM would be expected to selectively adhere to the hydrophobic coating, while charged microbial cells would have a reduced level of adherence.

Questions

In spite of the progress that has been made in understanding NTM ecology and epidemiology since recognition that the source of infection in humans was the environment (Wolinsky 1979), a number of unanswered questions remain. First, a risk analysis of human NTM infection is needed to establish the NTM numbers requiring action by utilities. However, before a meaningful risk analysis can be performed, we need to be able to distinguish virulent from avirulent strains. Unlike other water-borne pathogens whose genetic and physiologic traits leading to virulence are known, NTM virulence factors are unknown.

The recent report of isolation of rapidly growing NTM from a polluted site (Wang *et al.* 2006), suggests they may be significant members of microbial consortia involved in pollutant mineralization and nutrient cycling.

To date, there is little evidence to support this hypothesis, other than that single report and the fact that rapidly growing NTM are able to degrade a variety of hydrocarbons (Krulwich and Pelliccione 1979; Heitkamp *et al.* 1988; Burback and Perry 1993). It is mildly surprising that those reports, coupled with the hydrophobic nature of rapidly growing NTM cells (e.g. they would be expected to be concentrated at the hydrocarbon-aqueous interface), did not lead to further investigation. Of course, the relative slow growth of even the rapidly growing NTM reduces their ease of detection and cultivation, in spite of their unique features and, now, their well-established widespread distribution in the natural and engineered environment.

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