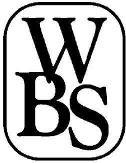

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# Nontuberculous mycobacteria in the environment

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## Environmental opportunistic mycobacteria

### *What are environmental opportunistic mycobacteria?*

Environmental opportunistic mycobacteria are those that are recovered from natural and human-influenced environments and can infect and cause disease in humans, animals, and birds. Other, less useful names for these mycobacteria are nontuberculous (however, they cause tuberculous lesions), atypical (to distinguish them from the typical *Mycobacterium tuberculosis*), and nontuberculous mycobacteria (NTM). Certain conditions (see Risk Factors, below) predispose to infection by the environmental opportunistic mycobacteria. The range of infections caused by environmental opportunistic mycobacteria is quite broad and includes skin infections (eg, *Mycobacterium marinum* and *Mycobacterium haemophilum*), cervical lymphadenitis (eg, *Mycobacterium avium*), joint infections (eg, *M avium* and *M intracellulare*), pulmonary infections (eg, *Mycobacterium kansasii, Mycobacterium avium,* and *Mycobacterium intracellulare*), bacteremia in AIDS (eg, *M avium*), and nosocomial infections (eg, *Mycobacterium fortuitum* and *Mycobacterium chelonae*).

The environmental opportunistic mycobacteria are normal inhabitants of natural waters, drinking waters, and soils. They can be isolated from biofilms, aerosols, and dusts. They have even been recovered from potting soils [1] and cigarettes [2]. If present in a water (eg, drinking water) or soil sample, they are not contaminants but rather are capable of persistence through growth.

### *Important environmental opportunist mycobacteria and their infections*

Environmental opportunistic mycobacteria include both slowly (Table 1) and rapidly growing (Table 2) mycobacteria. By far, the most important slowly growing species are *Mycobacterium avium* and *Mycobacterium intracellulare,* called the *M avium* complex (MAC). Although they are distinct species and can be identified and distinguished by DNA probes (AccuProbe, Gen-Probe, San Diego, CA), they are almost indistinguishable phenotypically. Not only are they the most common mycobacteria recovered from patients with disease, but also the incidence of infections caused by this group is increasing throughout the world.

Geographic differences in prevalence of mycobacterial species have been noted. For example, *M malmoense* is more common in Europe than in the United States. Periodicity of appearance also distinguishes the species. Infections caused by *M kansasii* and *M xenopi* are often clustered in both time and place, usually coinciding with the presence of the mycobacteria in drinking water.

It is important to point out that three species of rapidly growing mycobacteria, *Mycobacterium abscessus, Mycobacterium chelonae*, and *Mycobacterium fortuitum*, are opportunistic pathogens and not saprophytes. Members of these species are normal inhabitants of natural and drinking waters and, like their slowly growing relatives, are resistant to antibiotics and disinfectants. Not only can these mycobacteria cause pulmonary disease (see Risk Factors, below), but they are also found as causative agents of nosocomial infections (see Table 2).

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Table 1
Slowly growing mycobacteria and their infections

| *Mycobacterium* species | Infections | Reference |
|---|---|---|
| *Mycobacterium avium* | Pulmonary | [4,11] |
| | Cervical lymphadenitis in children | [10,11] |
| | Bacteremia in AIDS | [21] |
| | Tenosynovitis | [243] |
| *Mycobacterium genavense* | Bacteremia in AIDS | [244] |
| | Mycobacteriosis in birds | [162] |
| *Mycobacterium haemophilum* | Skin | [245] |
| | Bacteremia | [245] |
| | Cervical lymphadenitis | [246] |
| *Mycobacterium intracellulare* | Pulmonary | [4,11] |
| | Tenosynovitis | [243] |
| *Mycobacterium kansasii* | Pulmonary | [4,247] |
| | Skin | [248] |
| | Bacteremia in AIDS | [249] |
| *Mycobacterium malmoense* | Pulmonary | [250] |
| | Cervical lymphadenitis | [251] |
| | Bacteremia | [252] |
| *Mycobacterium marinum* | Skin | [253,254] |
| | Bacteremia | [255] |
| *Mycobacterium scrofulaceum* | Pulmonary | [4] |
| | Cervical lymphadenitis in children | [4,10] |
| | Skin | [256] |
| | Bacteremia | [257,258] |
| *Mycobacterium simiae* | Pulmonary | [4,259] |
| | Bacteremia | [260,261] |
| *Mycobacterium ulcerans* | Skin | [262,263] |
| *Mycobacterium xenopi* | Pulmonary | [4,105] |
| | Bacteremia | [264] |

In addition to the slowly growing mycobacterial species listed in Table 1, there have been reports of newly identified slowly growing species that have been associated with infection in patients. These newly described species are listed in Table 3. Because of the number of reports describing unidentified mycobacteria that are associated with disease (eg, 3), it is anticipated that the number of opportunistic pathogenic *Mycobacterium* species will continue to rise in the short term.

### *Risk factors for infection by environmental opportunistic mycobacteria*

Pre-existing pulmonary disease conditions such as silicosis, pneumoconiosis, and black lung and occupational exposure to dusts are risk factors for infection by environmental opportunistic mycobacteria [4,5]. Other risk factors include thoracic structural abnormalities [6], cystic fibrosis [5,7,8], and pulmonary alveolar proteinosis [9]. Young children with erupting teeth are at risk for cervical lymphadenitis caused by *M avium* [10,11]. Familial clusters of infections caused by environmental opportunistic mycobacteria have been reported [12–14]. Those reports were followed by evidence that mutations in the interferon $\gamma$ receptor gene [15–17], the interleukin (IL)-12 receptor gene [18], or the IL-12 gene [19] increased susceptibility of individuals to mycobacterial disease. Immunosuppression caused by malignancy [20], HIV-infection [21], and drug-treatment coincident with transplantation [22] are risk factors for infection by environmental opportunistic mycobacteria.

There are individuals who lack those predisposing risk factorss yet are infected with environmental opportunistic mycobacteria. The majority of the patients are elderly, slight women [23–25].

## Isolation, identification, and characterization of environmental opportunistic mycobacteria

### *Why the emphasis on the environment?*

Unlike infection by *Mycobacterium tuberculosis*, there is no evidence of person-to-person spread of the environmental opportunistic mycobacteria [4]. Recognition of that fact led to surveys to determine whether mycobacteria could be isolated from water and soil samples [26,27]. Evidence for the presence of mycobacteria in the environment was also demonstrated by the fact that greater than 60% of single county residents of the southeastern coastal United States showed evidence of prior infection by myco-

Table 2
Rapidly growing mycobacteria and their infections

| *Mycobacterium* species | Infection | Reference |
|---|---|---|
| *Mycobacterium abscessus* | Pulmonary | [5,265] |
| | Otitis media | [266] |
| | Injection abscess | [267] |
| *Mycobacterium chelonae* | Pulmonary | [5,268] |
| | Otitis media | [269] |
| | Peritonitis | [270] |
| | Bacteremia (AIDS) | [271] |
| *Mycobacterium fortuitum* | Pulmonary | [5,265] |
| | Surgery-associated | [272,273] |
| | Catheter-associated | [274] |
| | Bacteremia (AIDS) | [271] |

Table 3
Newly reported mycobacteria and their infections

| *Mycobacterium* sp | Infection | Reference |
|---|---|---|
| Slowly growing species | | |
| *M branderi* | Pulmonary | [3,275] |
| *M celatum* | Pulmonary (AIDS and non-AIDS) | [276] |
| *M conspicuum* | Bacteremia (AIDS) | [277] |
| *M heidelbergense* | Cervical lymphadenitis | [278] |
| *M interjectum* | Cervical lymphadenitis and pulmonary | [279] |
| | Kidney | [280] |
| *M intermedium* | Pulmonary | [281] |
| *M lentiflavum* | Pulmonary | [282] |
| *M magdeburgensis* | Pulmonary and lymphadenitis | [283] |
| *M triplex* | Bacteremia (AIDS) | [284] |
| *M tusciae* | Cervical lymphadenitis | [110] |

bacteria other than *M tuberculosis* [28,29]. Those studies employed a purified protein derivative prepared from a culture of a strain of *M intracellulare* (PPD-B; 28). The results prompted us to examine the geographic distribution of *M avium, M intracellulare,* and *M scrofulaceum* in eastern United States waters [30] and soils [31]. Since those initial surveys, others have demonstrated the presence of mycobacteria in environments throughout the world (below). Those studies have provided clues to the physiologic and genetic bases for the geographic distribution of mycobacteria and the pathways of human infection.

Because of the predominance of *M kansasii* and the *M avium* complex among patient isolates, most studies have focused on recovery or detection of those species; therefore, the absence of a particular species from any of the environmental samples recited below should not be considered evidence of its absence in a particular environmental compartment. Furthermore, increased susceptibility to disinfection and requirements for culture of some mycobacteria (eg, iron, mycobactin, temperature, and pH), may not have been met in all surveys.

## *Isolation, identification, and fingerprinting*

### *General problems*

The major difficulty in isolation of mycobacteria from environmental samples is their slow growth. Culture has been the standard for evidence of infection, in spite of the long time required for mycobacterial colonies to appear (ie, 2–8 weeks). The slow growth of mycobacteria results in overgrowth and contamination of colonies in specimens containing other microorganisms (eg, soil). Accordingly, those specimens are decontaminated by various treatments. The decontamination treatments rely on the relative resistance of mycobacteria to acid, base, or detergents. Decontamination reduces mycobacterial numbers and the sensitivity of detection, however [32]. Direct detection of mycobacteria is possible in samples with low microbial numbers such as drinking water [30,33] and aerosols [34].

Although there have been many comparisons of different culture isolation methods from environmental samples, general rules to guide isolation cannot be deduced because of differences in sample types, culture media for primary isolation, and differences in the geographic distribution of mycobacteria. Further, mycobacteria differ in their susceptibility to decontamination. For example, *M ulcerans* is quite sensitive to decontamination [35]. In addition, several species of *Mycobacterium* require specific substances or conditions for growth. For example, some form of iron is required for the growth of *M haemophilum* [36]; mycobactin is required for growth of *M avium* subspecies *paratuberculosis* [37]; and the presence of blood stimulates growth of *M genavense* [38]. Microaerobic conditions enhanced the growth of *M ulcerans* [35]. Low pH and microaerobic conditions promoted the growth of *M genavense* [39,40], and the combination of low pH and pyruvate enhanced the growth of *M malmoense* [41]. Growth temperatures below 37°C are optimal for the growth of *M haemophilum* (32°C) [36] and *M marinum* (30°C) [42].

### *Isolation from water*

In most studies of water, mycobacteria have been concentrated from large volumes of water (100–1000 ml) by either centrifugation [30,33] or filtration [43]. Following concentration, cell concentrates or filters can be directly placed on medium or decontaminated by a variety of methods [44]. Mineral acids (eg, HCl, $H_2SO_4$) and bases (eg, NaOH), organic acids (eg, oxalic acid), and detergents (eg, cetylpyridinium chloride, CPC) have been used as decontaminating agents [44]. All methods of decontamination reduce the numbers of mycobacteria as well as other bacteria and fungi, however [32]. Methods have been compared on the basis of the number and variety of mycobacteria recovered [45,46]. Methods yielding the highest number and variety of mycobacteria employ gentle decontamination with CPC [46]. CPC decontamination was successfully employed to isolate mycobacteria from biofilms in pipes in drinking water systems [33]. If possible, the best approach would be to avoid decontamination. Magnetic beads, coated with antimycobacterial antibody have been used to enrich and isolate mycobacteria from water [47].

*Isolation from soil*

Two major difficulties are encountered when attempting to isolate mycobacteria from soil, dust, or peat. First, these materials contain high numbers of faster growing microorganisms that can grow over and hide mycobacterial colonies. Second, mycobacteria adhere strongly to soil particles [32]. Increased numbers of mycobacteria can be isolated from soil by treating soil with polysaccharidases [48]. Although a great number and variety of decontamination methods have been devised to reduce the numbers of other microorganisms [32,44,45,49–52], all the methods reduce the numbers of mycobacteria [32]. It is even possible to use paraffin wax-coated glass slides to isolate mycobacteria and nocardia from soil by placing the slides in soil [53]. Consequently, as is the case for isolation of mycobacteria from sputum and feces after decontamination, the true number of mycobacteria cannot be established with certainty.

*Identification*

Classical culture, biochemical, and enzymatic methods are described for the identification of mycobacteria [54]. Those approaches take time, however, because of the slow growth of mycobacteria. As a result, rapid methods have been developed. Following isolation of colonies or broth cultures with suspected mycobacteria, the pattern of mycolic acids can be used for identification [55,56]. Amplification and analysis of patterns of restriction endonuclease digestion products of the 65-kilodalton heat shock protein gene (*hsp-65)* [57,58] has proven useful for identification of environmental opportunistic mycobacteria recovered from both patient and environmental samples. The 16S–23S rRNA gene internal transcribed spacer (ITS) [59], the 32-kilodalton protein gene [60], and *dnaJ* gene [61] also have been suggested for utility in identification of both slowly and rapidly growing mycobacteria. But the high degree of variation found within the *M avium dnaJ* gene [62] and the 16S-23S ITS [63] suggests that neither should be used for identification, but rather fingerprinting (see Fingerprinting, below). Commercial kits employing probes (Gen-Probe, San Diego, CA) or amplification (Roche Amplicor polymerase chain reaction (PCR) assay, Roche Diagnostic Systems, Inc, Branchburg, NJ) also are available.

DNA fragments or insertion sequences unique to a particular species can serve as targets for direct probe- or PCR-based identification methods. Quantitative PCR detection and enumeration may be possible using the most probable number (MPN)-PCR approach [64]. For example, *M ulcerans* contains a unique repeated sequence [65] and multiple copies of the unique insertion sequence (IS) *2404* [66] that can be used for identification. Arrays of either total DNA [67] or species-specific 16S rRNA sequences [68] have also been developed for identification of a wide range of *Mycobacterium* species.

*Fingerprinting*

A variety of methods of DNA fingerprinting are available for environmental opportunistic mycobacteria (Table 4). Some, such as serotyping and glycolipid

Table 4
Methods for fingerprinting environmental opportunistic mycobacteria

| Method | Genetic element | Applicable *Mycobacterium* species |
|---|---|---|
| Serotyping | | *M avium* complex [285] |
| Glycolipids | | *M malmoense* [286] |
| Multilocus enzyme electrophoresis | | All species<br>*M avium* complex [69] |
| Restriction fragment length polymorphism | | |
| Pulsed field gel electrophoresis | | All species<br>*M avium* [287]<br>*M paratuberculosis* [288]<br>*M haemophilum* [289]<br>*M fortuitum* [70]<br>*M chelonae* [71]<br>*M abscessus* [71] |
| Ribosomal RNA probe | 16S rRNA | *M malmoense* [290] |
| | 16S rRNA | *M fortuitum* [291] |
| | 5S rRNA | *M paratuberculosis* [292] |
| Repeated Sequence | $(GTG)_5$ | All species [293] |
| | MPTR | *M kansasii* [194] |
| | Unknown repeat | *M haemophilum* [294] |
| Insertion sequence | IS*1652* | *M kansasii* [194] |
| | IS*1245* | *M avium* [76] |
| | IS*901* | *M avium* [76] |
| | IS*900* | *M paratuberculosis* [295] |
| | IS*1081* | *M xenopi* [296] |
| | IS*1395* | *M xenopi* [297] |
| | IS*2404*, IS*2606* | *M ulcerans* [66] |
| Random amplified polymorphic DNA | | *M malmoense* [298]<br>*M abscessus* [109] |

profiling, are limited to a few species. Multilocus enzyme electrophoresis (MLEE) is applicable to all mycobacteria species, although to date only the relatedness of a large number of *M avium* isolates has been reported [69]. The most widely applicable method is restriction fragment length polymorphism (RFLP), examining either the large fragments separated by pulsed field gel electrophoresis (PFGE) or smaller fragments using IS or repeated sequences as hybridization probes (see Table 4). In the absence of known repeated genetic elements, PFGE has been successfully employed. For example, no repeated sequences (eg, insertion sequences) have been identified in the rapidly growing mycobacteria. Analysis of large restriction fragments by PFGE has, however, been successfully employed to study nosocomial outbreaks of these environmental opportunists [70,71]. The use of single primers in PCR reactions (RAPD) can lead to identification of species-specific sequences that can be used as probes for identification, as described for *M xenopi* [72] and for *M malmoense* [73] or RFLP analysis.

One fact that has become apparent from fingerprinting studies of the environmental opportunistic mycobacteria is that all species are quite heterogeneous. For example, typing has led to discovery of five subspecies of *M kansasii* [74] and at least three subspecies of *M avium* (*M avium* subspecies *avium*, *M avium* subspecies *silvaticum*, and *M avium* subspecies *paratuberculosis* [75]. Even within each *M avium* subspecies, there are a large number of individual types based on either IS *1245* typing [76] or differences in sequence of the 16S–23S internal transcribed spacer [63]. The broad diversity of types within each species suggests that care should be taken in employing primers for amplification of species-specific sequences. It is possible that some types may not have the complements to the primers, resulting in false-negative results.

## Habitats of environmental, opportunistic mycobacteria

### *Water*

#### *Natural waters in lakes, ponds, rivers, and streams*

A number of the environmental opportunistic mycobacteria have been isolated from natural waters [26,30,49,77–81] (Table 5). Highest numbers were reported in the acid brown-water swamps of the southeastern coastal United States [78] and waters draining from boreal forest soils and peat lands in Finland [79,82]. Consistent with the high numbers in waters from draining peat lands was earlier work showing high numbers of mycobacteria in *Sphagnum* vegetation [83] and the ability of mycobacteria, including *M avium* and *M intracellulare* to grow in the *Sphagnum* vegetation [83,84]. *M ulcerans* was detected in water samples using a combination of immunomagnetic enrichment and PCR [47] and from swamp and golf course irrigation water by PCR [85] within an area where an outbreak of *M ulcerans* occurred.

Table 5
Habitats of environmental opportunistic mycobacteria

| General habitats | Specific habitats |
|---|---|
| Natural waters | Lakes, ponds, estuaries, swamps, rivers |
| Drinking waters | Distribution systems, building systems |
| Biofilms | Pipes, tubing, filters |
| Soil | Soils, peat, potting soil |
| Aerosols | Water droplets, indoor aerosols, dusts |
| Equipment | Bronchoscopes, catheters |
| Moldy buildings | Water-damaged walls |

Changes in the types of mycobacteria in water have evidently occurred. Although *M scrofulaceum* has been recovered from water in the past [30,86], that is no longer the case [33,81]. That is consistent with the disappearance of *M scrofulaceum* as a causative agent of cervical lymphadenitis in children [10,11].

In a number of studies of natural waters, no correlation was found between the number of mycobacteria and fecal coliform counts [30,81]. Thus, sewage is not a source of environmental mycobacteria.

#### *Drinking water*

A wide variety of environmental opportunistic mycobacteria have been recovered from drinking water (see Table 5). In many studies, emphasis was placed on isolation and enumeration of *M avium* because of the high incidence of infection in AIDS patients [21]. Review of the frequency of recovery of all mycobacterial species indicates that recovery is not consistent from one sample to the next in a single system [33,87,88]. Thus, repeated samples are required for proof of mycobacterial presence in a drinking water system.

Representatives of *M avium*, *M intracellulare,* or the *M avium* complex have been isolated from drinking water [26,33,43,89–95], public bath waters [96], hospital water systems [86,97,98], and water supplies of hemodialysis centers [99]. In addition, other mycobacteria have been recovered from drinking water including *M kansasii* [88,92,100–103], *M marinum* [104], *M malmoense* [92], *M scrofulaceum* [86], *M xenopi* [92,94,102–107], *M fortuitum* [70,93,99, 100,108], *M abscessus* [71,109], and *M chelonae*

[71,92,99,100,]. One of the newly described species of mycobacteria, *M tusciae* has also been isolated from tap water [110]. Pseudoinfections, caused by the presence of environmental opportunistic mycobacteria in laboratory water supplies used for the preparation of solutions for detection or isolation of mycobacteria, have been reported [86,98,107]. As was the case for natural waters, no correlation was found between mycobacterial numbers and fecal coliforms or other microbiologic or chemical indicators of water quality in swimming pools [111] or drinking water [33].

Environmental opportunistic mycobacteria were not recovered from bottled water (0 of 31 samples) in two independent studies [89,112]. This is consistent with the fact that mycobacteria were seldom recovered from ground waters [33,113].

*Biofilms*

Biofilms may be important sources of environmental opportunistic mycobacteria and, perhaps, the basis for their persistence in drinking water systems (see Table 5). Mycobacteria, including *M avium* and *M intracellulare*, have been isolated from biofilms in drinking water distribution systems [33,114]. The number of mycobacteria in biofilms can be as high as 10,000–100,000 colony forming units per $cm^2$ [33,114]. Caution must be taken in interpreting those numbers because surface scraping is likely not quantitative and disinfection will reduce mycobacterial numbers. In spite of those caveats, considering the surface area of water distribution systems in the hundreds of miles of pipe, mycobacterial numbers in suspension may be maintained not by the introduction of mycobacteria from source water but from their entrainment from biofilms. This is consistent with the observation that drinking water distribution systems whose ground water source lacks mycobacteria have a resident population of mycobacteria.

Mycobacteria readily form biofilms and, because of their hydrophobicity and metal-resistance (below), may be pioneers of biofilm formation. *M kansasii* biofilms on silicone tubing initially appeared after 3 weeks following insertion of the tubing into a warm water distribution system (ie, 35–45°C) that contained *M kansasii* [115]. By 10 months, the biofilms contained $2 \times 10^5$ colony forming units/$cm^2$ [115]. *M fortuitum* biofilms of almost $10^6$ colony forming units per $cm^2$ were formed after 2 hour incubation at 37°C on the surface of silicone [116]. The rapid formation and high number of cells in the *M fortuitum* biofilm was likely caused by the high number of cells in suspension ($10^8$ colony forming units/ml) [116].

Mycobacteria can colonize water-filtration devices. In-line, carbon filter units impregnated with silver were shown to be colonized by *M avium* and *M fortuitum* [117]. In fact, they supported growth of *M avium* [117]. Colonization and growth of *M avium* was likely caused by its ability to grow in drinking water [33,99,118], its metal resistance [119], and hydrophobicity [120]. Mycobacteria have also been shown to adhere to cellulose diacetate membranes in reverse osmosis systems for water treatment [121].

The presence of environmental opportunistic mycobacteria in biofilms can directly impact patient health. *M avium* complex sepsis in a patient was linked with colonization of a central venous catheter [122]. Biofilms in water lines in dental drilling and cleaning devices have also been shown to contain mycobacteria, including *M chelonae* [46].

*Soil*

Soils, like natural waters, yield a wide variety and high numbers of environmental opportunistic mycobacteria (see Table 5). Unfortunately, many of the studies were narrowly focused on a single species to identify its environmental source. Published reports document the recovery from soils of: *M kansasii* [123,124], *M avium* complex [27,31, 49,124–127], *M malmoense* [128], and *M fortuitum* [27,123,124]. Consistent with the recovery of mycobacteria from soil are reports of isolation of *M kansasii*, *M avium* complex, and *M fortuitum* from house dusts [124, 129,130].

Boreal coniferous forest soils were shown to have very high numbers of environmental opportunistic mycobacteria [131]. Consistent with that observation was recovery of isolates of *Mycobacterium* species from 89%, *M avium* complex from 55%, and *M avium* from 27% of samples of peat-rich potting soil [1]. Mycobacteria, including *M avium* and *M intracellulare*, have also been recovered from anaerobic river sediments [31,132].

*Aerosols*

Although there have been few reports documenting recovery of mycobacteria from aerosols (see Table 5), such studies offer a promising approach for following the transmission of environmental opportunistic mycobacteria. Aerosols can be collected as colony forming units using the Andersen impact sampler [133]. Water droplets ejected from the surface of waters containing mycobacteria can be sampled by inverting a Petri dish containing an agar medium suitable for mycobacteria growth 10 cm above the

water surface [34]. Unless there is substantial dust in the air, mycobacterial colonies can be recovered after incubation and identified. If fungi overgrow possible mycobacterial colonies, malachite green (final concentration of 0.005%) can be added to the medium to suppress fungal growth without inhibiting mycobacterial growth. Because the volume of ejected droplets can be measured [34], it is possible to calculate the concentration of mycobacteria in droplets and thereby determine whether they are concentrated in the ejected droplets by comparison with the concentration in the suspension [134].

Members of the *M avium* complex have been isolated from ejected droplets and aerosols generated by a natural river [34]. In laboratory experiments, it was shown that *M avium* and *M intracellulare* cells were concentrated by factors of 100–5000 in droplets ejected from suspensions of cells [134]. Mycobacteria including members of the *M avium* complex have also been recovered from dusts formed by airflow across rivers, agricultural fields, and parks [135].

There are a number of reports of hypersensitivity pneumonitis in different occupations that likely involve aerosolization of environmental opportunistic mycobacteria. Hypersensitivity pneumonitis has been found in machine tool operators exposed to metalworking fluid aerosols [136,137]. Although mycobacteria have not been isolated from metalworking fluid aerosols, the fact that hypersensitivity pneumonitis develops following attempts to disinfect the metalworking fluid and mycobacteria were isolated from the disinfected metalworking fluid samples [137] strongly suggests that mycobacteria have a role in that occupational disease. Mycobacteria are resistant to the quaternary ammonium compounds used for disinfection of metalworking fluid [138]. Pneumonitis has also been observed in lifeguards in an indoor swimming pool [139]. Here again, mycobacteria were not directly linked to the outbreak of disease in lifeguards, but a variety of mycobacteria have been isolated from swimming pools [111] and whirlpool therapy baths [140], and mycobacteria are very resistant to chlorine [141]. It would be expected that aerosols would be rich in mycobacteria because of their concentration in ejected droplets [134].

### Moldy buildings

Mycobacteria were recovered from water-damaged, moldy buildings [142], and *M avium* from a water-damaged building during demolition [143]. These observations alone are not surprising in light of the presence of mycobacteria in natural and drinking waters. Furthermore, the mycobacterial isolates from the moldy buildings proved to be potent inducers of inflammatory responses [143]. These observations suggest that respiratory disease syndromes associated with exposure to indoor environments in water-damaged, moldy buildings may be associated with mycobacteria or their metabolites.

### Instruments

There have been a variety of reports documenting the presence of mycobacteria in bronchoscopes. As noted above, biofilms in water lines in dental drilling and cleaning devices contain a variety of mycobacteria [46]. Bronchoscopes have been shown to be contaminated with *M avium* [144], *M intracellulare* [145], *M xenopi* [146], and *M chelonae* [144,147,148]. In most cases, contamination was suspected because of unusual increases in the isolation of a particular mycobacterial species. In retrospective studies, the presence of the *Mycobacterium* species was caused by inadequate decontamination of the bronchoscope following its use with an infected patient [144–148]. The source of the mycobacteria in the contaminated bronchoscopes was identified as a patient in one report [145] and caused by the presence of the mycobacteria in the hospital water system in two others [144,146]. The persistence of the mycobacteria in bronchoscopes is undoubtedly caused by their resistance to disinfectants [149–152].

### Food

Mycobacteria were almost absent in 100 samples of meat, 121 vegetables, 138 dairy products, and 38 eggs [1]. In fact only 2 of the 397 food samples yielded a *M avium* complex isolate, and only 12 (3%) of the food samples yielded a *Mycobacterium* species isolate [1]. Quite possibly, the origin of the mycobacteria on the seven vegetable samples yielding *Mycobacterium* spp. was water used to wash the vegetables. Raw milk samples yielded isolates of *M kansasii*, *M avium, M intracellulare*, and *M fortuitum* [153–155], and 7% of retail milk samples were shown to contain *M paratuberculosis* based on IS900-PCR amplification [156].

### Animals and birds

Although environmental opportunistic mycobacteria have been isolated from wild and domestic birds and animals, it is not always clear, as is the case for plants, whether the source is the bird or animal (ie, zoonotic) or the environment. There is one case where infection in animals has been traced to the

presence of mycobacteria in water. Simian virus-infected macaques were shown to have acquired *M avium* infection from potable water [157]. *M avium* has been recovered from tuberculous cervical and mesenteric lymph nodes of pigs [158–161] and domestic fowl [158]. *M avium, M fortuitum,* and *M genavense* have been recovered from captive exotic birds [162–165] and from wild birds [166,167a]. In one case, investigators made note of an association between the presence of *M avium* in starlings and the high incidence of tuberculous lesions in swine [166].

*Miscellaneous samples*

In addition to the environmental compartments listed above, a number of other possible reservoirs for mycobacteria have been sampled. *M avium* has been isolated from cigarette tobacco, cigarette filters, and the paper surrounding tobacco in cigarettes [2]. A variety of mycobacteria, including *M avium* and *M intracellulare*, have been isolated from *Sphagnum* vegetation [77,78]. Unidentified rapidly growing mycobacteria have been recovered from soils in the presence of legume root-nodule bacteria [167b].

Mycobacterial contamination of both plant and animal cell tissue cultures has been reported [168–170]. Because laboratories seldom test for evidence of mycobacteria contamination and the environmental opportunistic mycobacteria are disinfectant- and antibiotic-resistant, cell culture contamination might be more widespread than the few reports might indicate. In the case of plant tissue culture, it was not known whether the mycobacteria were present on the original plant tissue (ie, epiphytes) and survived preparation of the tissue for culture or, alternatively, were introduced through water used to prepare media [170]. *M avium* complex isolates have been recovered from pine-bark–based mulch used as bedding material for broiler chickens [171] and pigs [172].

## Physiologic ecology of environmental opportunistic mycobacteria

*Structural characteristics*

Environmental opportunistic mycobacteria are called acid-fast because they retain the dye fuchsin in phenolic solution after exposure to dilute solutions of mineral acids [173]. Dye retention is caused by the presence of long-chain lipids (mycolic acids) in the outer layers [174]. Members of the genus *Mycobacterium* have a thin peptidoglycan-based cell wall and both a cytoplasmic and outer membrane [174]. The fluidity of the outer membrane is determined by the structure of the mycolic acids [175,176]. The hydrophobicity of the cell surface coupled with the existence of a single porin [177], contribute directly to the low permeability of mycobacteria [178], and their slow growth rates and resistance to disinfectants and antibiotics [174] (Table 6). A capsule consisting of protein and carbohydrate, dependent on the species and the stage of growth and conditions [179], also surrounds mycobacterial cells. That capsule, called the electronic transparent zone, is found in mycobacterial cells within infected macrophages and tissue and may contribute, in part, to their ability to survive and grow intracellularly [180].

*Cell surface hydrophobicity and charge*

Environmental opportunistic mycobacteria are among the most hydrophobic of cells [181]. This undoubtedly contributes to their ability to be aerosolized [134], form biofilms [115], phagocytoze rapidly [181], as well as their slow growth rate [178], aggregation [134], impermeability [178], and resistance to antimicrobial agents [174] (see Table 6). Mycobacterial hydrophobicity can be measured easily by adherence

Table 6
Physiologic characteristics of environmental opportunistic mycobacteria relevant to their epidemiology

| Characteristic | Consequence |
|---|---|
| Impermeable cell membrane | Slow growth rate<br>Antibiotic-, disinfectant-, and metal-resistance |
| Hydrophobic cell surface | Reduced uptake of hydrophilic molecules<br>Antibiotic, disinfectant, and metal-resistance |
| Acidophilic (pH 5-6) | Present in acidic environments<br>Survive passage through stomach |
| Growth in fresh and brackish water | Increase in number to end of distribution system<br>Increase in number in environment |
| Growth at low oxygen levels | Growth in oxygen-limited habitats<br>Growth in tissue |
| Growth over wide temperature range | Increase in number in environment |
| Intracellular growth | Growth in protozoa and amoebae<br>Survive in cysts<br>Growth in macrophages |

to hexadecane [182]. The difference in hydrophobicity of colony variants of *M avium* strains [120] may contribute the their differences in antimicrobial susceptibility and virulence. The opaque colony variants were more antibiotic-susceptible, less virulent, and more hydrophilic than were their isogenic, antibiotic-resistant, virulent transparent variants [120].

The surface charge of *M avium, M intracellulare*, and *M scrofulaceum* cells is quite different from other bacteria. Cells of representatives of those three species were electronegative above a pH of between 3.5–4.5 [183]. This means that, at near-neutral pH values, cells of these mycobacteria are rather strongly negatively charged and would be expected to attract positively charged compounds (eg, cations) and repel like-charged compounds (eg, anions). This may be related to the ability of *M avium* and *M scrofulaceum* to grow at pH 6 under conditions of $Mg^{2+}$ limitation [184]. Only under rather acidic conditions (eg, pH 4) would these cells lack a charge. This may explain, in part, the fact that *M avium, M intracellulare*, and *M scrofulaceum* exhibit optimal growth at acidic pH values [185–187].

### *Genomics and genetic variation*

Extrachromosomal genetic elements, including plasmids and bacteriophage, have been described in environmental opportunistic mycobacteria. The absence of reports of such elements in individual species should not be taken as indicative of their absence. Simply, no one has looked. Plasmids have been reported in members of the *M avium* complex and *M scrofulaceum* [188–190], *M fortuitum* [191,192], and *M chelonae* [193]. Large linear plasmids have been isolated from *M xenopi, M branderi,* and *M celatum* [194].

*M avium* complex plasmids are likely to contribute significantly to the genetic makeup of that group because the plasmids are large (eg, 15–300 kb) and single strains can harbor as many as six individual plasmids that comprise 30% of the DNA of the strain [189]. The presence of identical plasmids in *M avium, M intracellulare*, and *M scrofulaceum* [189,190], or common identical fragments of plasmid in *M xenopi, M branderi*, and *M celatum* [194], indicates that the plasmids are transmissible between strains. Isolation of mycobacterial plasmids is difficult because cells are difficult to lyse and still retain plasmid integrity [188–190]. Furthermore, mycobacterial plasmids appear to be unusually stable and difficult to cure [195]. The unusual stability of mycobacterial plasmids has restricted identification of plasmid-encoded genes. In spite of these difficulties, *M avium* plasmids have been shown to encode for a restriction and modification enzyme [196], and for mercury- [195] and copper-resistance [197]. Morpholine-degradation in a strain of *M chelonae* was shown to be plasmid-encoded [193].

Bacteriophages whose hosts are mycobacteria (ie, mycobacteriophages) have been isolated and described [198]. A major focus has been on bacteriophage infecting *M avium* because of the importance of that species in AIDS-related bacteremia. Both lytic [199] and lysogenic [200,201] bacteriophage infecting *M avium* complex cells have been reported. The host range for mycobacteriophages may be quite wide, based on the fact that most phage can form plaques on members of the *M avium* complex and on the rapidly growing saprophyte *Mycobacterium smegmatis* [199,201,202]. Bacteriophage able to form plaques on *M smegmatis* and *M kansasii* were described by Coene et al [198]. Mycobacteriophages have been employed for typing isolates of the *M avium* complex [199] and, more recently, as cloning vectors [200,203]. Mycobacteriophages D29 and B4 were shown to result in a change of colony type from rough to smooth on formation of lysogens of *M smegmatis* [204].

### *Growth characteristics*

Mycobacteria are slow growing. Slowly growing mycobacteria require at least 7 days and for some species and strains 28 days for the appearance of turbidity or formation of visible colonies. Although described as rapidly growing, *M fortuitum, M chelonae,* and *M abscessus* still take 3–5 days to form turbid cultures in broth or 1–2 mm diameter colonies at 37°C. Cells of *M avium* and *M intracellulare* transferred to fresh medium display biphasic, exponential growth in which growth rates in the first 2 days can be as high as 2 generations per day [205]. Slow growth rate must not be thought to imply that mycobacteria are metabolically sluggish. Mycobacteria simply expend a great deal of energy in synthesizing long chain fatty acids and lipids. In addition to directing energy toward synthesis of the lipid-rich, complex envelope, mycobacteria have a relatively impermeable membrane and hydrophobic surface that limit transport of hydrophilic nutrients [174,178]. The presence of either one (ie, slowly growing) or two (ie, rapidly growing) rRNA cistron [206,207] also limits the rate of protein synthesis and mycobacterial growth.

Growth rate is an important factor in mycobacterial epidemiology and antimicrobial resistance. Slow growth rates mean that mycobacteria die very slowly, if at all, under natural conditions [118] or when

exposed to chlorine [141] or antibiotics [208,209]. Thus, the environmental opportunistic mycobacteria have traded rapid growth for resistance.

Measurement of mycobacterial growth is subject to a variety of problems. First, cells grow slowly and thus, are subject to contamination. Second, mycobacterial cells aggregate during growth, preventing accurate measurement of growth rates by turbidity or cell counts either by microscope or by colony formation. Although addition of a detergent such as Tween 80 (0.1–1.0%) can reduce aggregation, it influences susceptibility of cells to antimicrobial agents [174]. Third, colony counts on agar media often are not equal to cell counts. Cell counts can be 10 times higher than colony counts, especially in the latter stages of growth or in stressful environments such as within protozoa [210].

### *Metabolism and metabolites*

Environmental opportunistic mycobacteria not only survive but grow in their environmental reservoirs. *M avium, M intracellulare*, and *M scrofulaceum* grew in natural waters over a wide range of temperatures and salinity [118]. *M chelonae* grew in commercial distilled water [211]. Mycobacterial numbers, including *M avium*, increased in proportion to the concentration of assimilable organic carbon in drinking water distribution systems from the treatment plant to the end of the pipelines [33].

Environmental opportunistic mycobacteria can use a wide variety of carbon and nitrogen for growth and nutrition. For the most part, knowledge of carbon and nitrogen sources utilized by mycobacteria has come from studies whose objective is the identification of species by classic means [212,213]. Because these growth studies were performed using minimal defined media [212], these opportunists are not, with few exceptions, auxotrophs. Carbon sources including acetate, glycerol, glucose, pyruvate, citrate, and propanol serve as sole carbon sources [212]. Amino acids, ammonia, nitrate, and nitrite serve as nitrogen sources [214]. Enzymes of the Embden-Meyerhof-Parnas pathway, the tricarboxylic acid cycle, and the glyoxylate shunt have been found in those environmental opportunistic mycobacteria that have been examined [215–217]. Using fluorogenic substrates, a wide variety of hydrolytic enzymes have been shown to be produced by mycobacteria [213]. Utilization of a wide range of carbon and nitrogen sources and production of a variety of hydrolytic enzymes [216] is consistent with the ability of environmental opportunistic mycobacteria to grow and survive in different environmental compartments.

A number of mycobacteria are capable of degradation of important pollutants that are relatively resistant to degradation. The hydrophobicity and resistance to antimicrobial agents (eg, metals and detergents) of mycobacterial cells may contribute to their ability to degrade these hydrocarbons. The compounds include: vinyl chloride [218], polychlorinated phenolics [219], polycyclic aromatic hydrocarbons [220,221], and dioxane [222]. It appears that mycobacteria may be more important agents of carbon and nitrogen cycling in the environment than hitherto imagined. Polluted sites may also serve to select for mycobacteria and pose a risk to humans, animals, and birds.

In addition to the extracellular capsular material [179], environmental opportunistic mycobacteria excrete a variety of extracellular enzymes and proteins [223]. It is likely that some of these extracellular metabolites are highly immunogenic [143].

### *Conditions of growth: pH, temperature, salinity, and oxygen*

Environmental opportunistic mycobacteria grow over a rather wide range of pH values. Growth optima for almost all mycobacteria are at acidic pH values (see Table 6). There is little growth at alkaline pH values above 7.5. The optimal pH for growth of *M kansasii, M marinum, M avium, M intracellulare,* and *M xenopi* is between 5.0 and 6.5 [185–187]. It is likely that both *M genavense* [39,40] and *M malmoense* [41] grow optimally at acidic pH because their isolation frequencies are increased by use of low pH media. The pH optima for growth of the rapidly growing mycobacteria, *M fortuitum, M chelonae*, and *M abscessus,* is also between 5.0 and 6.5 [185,187]. It is not known whether the cell surface charge of other environmental opportunists is electronegative above pH 4 as it is for *M avium, M intracellulare*, and *M scrofulaceum* [183].

Environmental opportunistic mycobacteria are capable of growth over a wide range of temperatures (see Table 6). *M avium* and *M xenopi* can grow at 45°C, which explains their recovery from hot water systems [97,106]. *M avium, M intracellulare,* and *M scrofulaceum* grow, albeit slowly, at temperatures as low as 10°C [118]. The ability of *M avium* and *M xenopi* to grow over a temperature range of 10°–45°C suggests that those species must be able to modify the composition of their membrane lipids to maintain a fluid, yet intact, permeation barrier [175,176,224,225]. Furthermore, one reason for the difficulty in isolation of mycobacteria from environmental samples may be that the sudden shift from low temperature to 37°C may either retard colony

formation or actually kill cells because the membrane is too fluid [224,225]. Growth temperatures below 37°C are optimal for the growth of *M haemophilum* (32°C) [36] and *M marinum* (30°C) [42], consistent with their clinical presentation as skin infections in most patients.

Environmental opportunistic mycobacteria have been isolated from fresh, brackish, and salt water [30] (see Table 6). Furthermore, *M avium, M intracellulare*, and *M scrofulaceum* grow in both fresh and brackish waters up to a salt concentration of 2% (expressed as gm NaCl/100 ml) [118]. The ability of this group to grow over this range of salt concentration is consistent with their isolation from estuaries [30,31]. A variety of mycobacteria including strains of *M kansasii, M marinum, M avium, M xenopi*, and *M fortuitum* have been shown to survive in ocean water (ie, Atlantic or Mediterranean) for more than 3 months [226]. Some strains survived up to a year [226].

Although mycobacteria are considered obligate aerobes, that is likely not the case for most all of the environmental opportunists. First, they are isolated from a variety of habitats whose oxygen levels are low (see Table 6). Higher numbers are recovered from the oxygen-deficient ends of water distribution systems than from the mid-points or immediately downstream from the treatment plant [33] Further, numbers of mycobacteria in both swimming pools and whirlpool baths were inversely correlated with oxidation-reduction potential [140]. Higher numbers were present in pools and baths of low oxidation-reduction potential. *M avium, M intracellulare,* and *M scrofulaceum* numbers in southeastern United States' rivers [31] and southeastern coastal acid-brown water swamps were highest in samples of low oxygen content [178]. Second, *M kansasii, M intracellulare,* and *M fortuitum* were shown able to adapt to growth under oxygen-limited conditions in laboratory medium [227]. Significantly, mycobacteria grown under oxygen limitation lost acid-fastness, pigmentation, and the ability to transport iron into cells [227]. Loss of acid-fastness means that staining might miss mycobacteria from low-oxygen habitats, including tissue and distal portions of drinking water distribution systems. Furthermore, the cells became sensitive to malachite green [227]. Because that dye is present in most mycobacterial media, it is possible that mycobacteria might be missed by culture. Because of the presence of a variety of compounds in the medium [227], it is not known whether electron acceptors other than oxygen (eg, nitrate) were used by the mycobacteria to support the growth. Complete oxygen depletion during growth results in a reversible dormant state in *M smegmatis* [228]. Furthermore, *M smegmatis* cells in the dormant stage became sensitive to the antibiotic metronidazole and resistant to ofloxacin; exactly opposite compared with cells grown in oxygen [228].

*Antibiotic-, disinfectant-, and heavy-metal resistance*

Environmental opportunistic mycobacteria are resistant to a broad spectrum of antibiotics and disinfectants (see Table 6). Thus, there has been the need to develop antimycobacterial antibiotics. Broad spectrum resistance to antibiotics and disinfectants is caused, in part, by the hydrophobicity, impermeability, and slow growth of mycobacteria [174, 178]. Broad-spectrum resistance has been related to the presence of a member of a family of membrane-associated transport proteins that mediate efflux of antibiotics (eg, quinolones) from mycobacterial cells [229,230]. In addition, antibiotic-specific mechanisms of resistance have been identified including the presence of β-lactamases [231,232], aminoglycoside acetyltransferases [233], and genes for tetracycline resistance [234]. Because of the innate high-level resistance of mycobacteria to antibiotics, the identification of specific resistance mechanisms is difficult.

Environmental opportunistic mycobacteria whose susceptibility to chlorine has been measured (eg, *M kansasii, M avium*, and *M fortuitum*) are all extremely resistant [141,211,235,236] (see Table 6). In fact, *M avium* is approximately 1000 times more resistant to chlorine than is *Escherichia coli,* the standard for drinking water disinfection [141]. Disinfection resistance extends to other agents used for drinking water treatment, namely chloramine, chlorine dioxide, and ozone in *M avium* [141]. The resistance is so high that mycobacterial numbers are not substantially reduced by disinfectant exposure, and they are present in the distribution system [33,89].

Mycobacteria are also resistant to disinfectants used for sterilization of surfaces and instruments. Disinfectant resistance is the basis for various decontamination regimens. In spite of their relative resistance, decontamination can reduce the number of viable mycobacteria [32]. Compared with other bacteria, mycobacteria are relatively resistant to benzalkonium chloride [151], cetylpyridinium chloride [237], quaternary ammonium compounds [138], and phenolic- and gluteraldehyde-based disinfectants [149,150,152]. Resistance to some disinfectants and detergents may be related to the presence of an efflux protein that mediates efflux of antibiotics and chemicals from mycobacterial cells [229]. As was pointed out above in the discussion of mycobacterial contamination of instruments that come in contact with patients, disinfectants

are effective against mycobacteria when used at the appropriate concentration [149] and are not reused.

*Intracellular growth in phagocytic cells*

Like *M tuberculosis, M kansasii, M avium*, and *M intracellulare* are intracellular pathogens. In addition to growth in human phagocytic cells, *M avium* and *M intracellulare* are capable of growth in phagocytic protozoa and amoebae. *M avium* has been shown capable of growth in *Acanthamoeba polyphaga* [238] and *A castellani* [239] and *M avium* and *M intracellulare* (but not *M scrofulaceum*) strains replicate in *Tetrahymena pyriformis* [210]. Cells of *M avium* and *M intracellulare* survive encystment and germination of *A polyphaga* [238] and *T pyriformis* [210]. Most interesting are observations that the virulence of *M. avium* is increased by virtue of growth in either amoebae [239] or protozoa (Falkinham unpublished observation). Thus, numbers and virulence of *M avium, M intracellulare*, and presumably other environmental opportunistic mycobacteria are increased by their presence in habitats occupied by protozoa and amoebae (see Table 6). Exposure to protozoa- or amoebae-grown *M avium* may be a risk factor for cervical lymphadenitis in children. Furthermore, it is intriguing to speculate that selection for intracellular survival of mycobacteria led to their ability to survive in human, animal, and bird phagocytic cells.

## Pathways of infection by environmental opportunistic mycobacteria

Infection by environmental opportunistic mycobacteria is a consequence of overlaps between human and mycobacterial environments. Drinking water has been shown to be at least one source of human and animal *M avium* infection. DNA fingerprints showed that water isolates of *M avium* were identical to those recovered from AIDS patients who either drank or were exposed to the waters [240]. Simian immunodeficiency virus-infected macaques were shown to be infected with *M avium* isolates whose fingerprints were identical to those of *M avium* strains isolated from the water they drank [157]. The link between water and animal infection is strong in this latter case because there was a single controlled source of water for the macaques. The high incidence of *M avium* infection in Finnish AIDS patients [241] correlated with very high numbers of *M avium* in natural and drinking water samples in Finland [81]. Coincidences between the outbreak of mycobacterial pulmonary disease and isolation of either *M xenopi* [102,106] or *M kansasii* [102,103] from drinking water also provides evidence that the environment is the source of infection. Such exposures could occur through either ingestion of water or exposure to mycobacterial-laden aerosols (eg, showers).

Mycobacterial cervical lymphadenitis in children could result from ingestion of either drinking water or natural waters. Currently, the majority of cases of mycobacterial cervical lymphadenitis is caused by *M avium*. Until approximately 1975–1980, however, the majority of cases were caused by *M scrofulaceum* in the United States [10]. Because it is unlikely that children have changed in that time period, the basis for that shift could be a change in behavior of children (eg, fluoridation as *M scrofulaceum* is chlorine-sensitive compared with *M avium*) or a change in mycobacterial ecology brought about by human activities (chlorination) [242]. *M scrofulaceum* has not been recovered from waters in recent surveys of drinking water in the United States [33,89].

Another possible overlap resulting in exposure to mycobacteria or their metabolites exists in water-damaged, moldy buildings. Water is often the limiting factor controlling microbial growth in indoor environments. Introduction of water, with its indigenous mycobacterial flora, would be expected to result in microbial proliferation. In fact, mycobacteria were recovered from material collected in a water-damaged, moldy building whose occupants reported nasal and eye irritation [142]. Furthermore, mycobacteria isolated from moldy buildings are capable of eliciting inflammatory responses [143]. Thus, exposure to either mycobacterial cells or metabolites may lead to allergic symptoms.

Certain occupations and leisure activities may also result in exposure to mycobacteria. Occupational exposures include work involving fishing and aquaculture, indoor swimming pools or therapy baths, and metalworking fluid. Leisure activities involve the maintenance of aquaria (eg, tropical fish) and exposure to aerosols or dusts containing mycobacteria. Leisure activities involving dust exposures include gardening and perhaps the use of peat-rich potting soils.

## Summary

It is likely that the incidence of infection by environmental opportunistic mycobacteria will continue to rise. Part of the rise will be caused by the increased awareness of these microbes as human pathogens and improvements in methods of detection and culture. Clinicians and microbiologists will continue to be challenged by the introduction of new

species to the already long list of mycobacterial opportunists (see Table 3). The incidence of infection will also rise because an increasing proportion of the population is aging or subject to some type of immunosuppression. A second reason for an increase in the incidence of environmental mycobacterial infection is that these microbes are everywhere. They are present in water, biofilms, soil, and aerosols. They are natural inhabitants of the human environment, especially drinking water distribution systems. Thus, it is likely that everyone is exposed on a daily basis.

It is likely that certain human activities can lead to selection of mycobacteria. Important lessons have been taught by study of cases of hypersensitivity pneumonitis associated with exposure to metalworking fluid. First, the implicated metalworking fluids contained water, the likely source of the mycobacteria. Second, the metalworking fluids contain hydrocarbons (eg, pine oils) and biocides (eg, morpholine) both of which are substrates for the growth of mycobacteria [53,193]. Third, outbreak of disease followed disinfection of the metalworking fluid [136,137]. Although the metalworking fluid was contaminated with microorganisms, it was only after disinfection that symptoms developed in the workers. Because mycobacteria are resistant to disinfectants, it is likely that the recovery of the mycobacteria from the metalworking fluid [137] was caused by their selection.

Disinfection may also contribute, in part, to the persistence of *M avium* and *M intracellulare* in drinking water distribution systems [33,89,240]. *M avium* and *M intracellulare* are many times more resistant to chlorine, chloramine, chlorine dioxide, and ozone than are other water-borne microorganisms [141,236]. Consequently, disinfection of drinking water results in selection of mycobacteria. In the absence of competitors, even the slowly growing mycobacteria can grow in the distribution system [33]. It is likely that hypersensitivity pneumonitis in lifeguards and therapy pool attendants [139] is caused by a similar scenario.

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