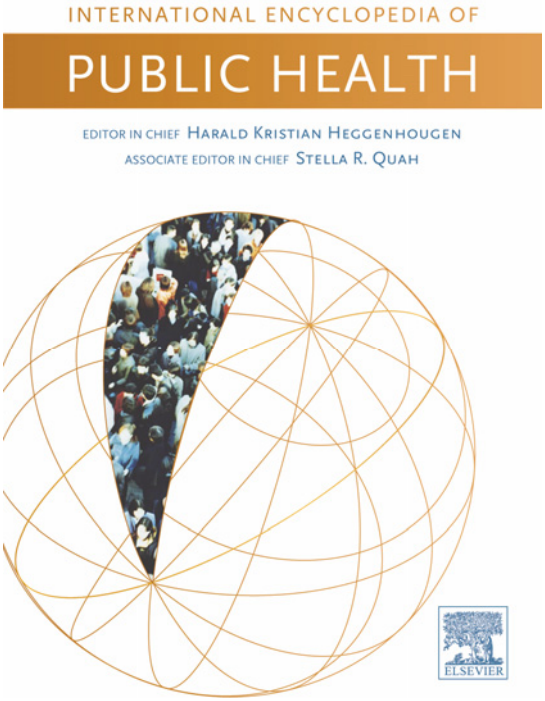

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Environmental Opportunistic Mycobacteria

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The Environmental Mycobacteria

The environmental mycobacteria (EM) represent a large group of opportunistic pathogenic bacteria whose source of infection (reservoir) is the environment. The global burden of these organisms is unknown, although an approximation can be made from U.S. data. While infections caused by EM are not on the Centers for Disease Control and Prevention (CDC) reportable list, current estimates place the incidence of disease at four cases per 100 000, leading to an estimate of 12 000 new cases annually in the United States. Because of their relative antibiotic resistance, multidrug treatment regimens can be more than 5 years in duration. Thus, the burden of disease due to EM is quite high.

Environmental opportunistic mycobacteria are free-living bacteria with a wax- and lipid-rich outer membrane that is a major determinant of their ecology and epidemiology. *Mycobacterium* was first named as a genus and the type species, *M. tuberculosis*, was deposited in 1896. When clinicians isolated acid-fast bacteria from patients or animals that did not characteristically match *M. tuberculosis*, these isolates were assumed to be mutant strains, and thus termed atypical mycobacteria, a term still, unfortunately, in use today. As it was realized that other species of *Mycobacterium* existed and these were named, other terms came into usage, such as MOTT (mycobacteria other than tuberculosis) and NTM (nontuberculous mycobacteria, which is technically inaccurate because many EM can form tubercules in the lung during infection). As the names clearly indicate, philosophically *M. tuberculosis* was still considered the 'normal' *Mycobacterium* species and others were secondary. However, at present the genus contains over 100 species, with the vast majority being free-living, opportunistic pathogens, unlike members of the *M. tuberculosis* complex that are obligate pathogens with a restricted host range that fail to survive in the environment. Further, the EM have relatively large genomes, carry plasmids, show evidence of horizontal gene transmission and physiologic flexibility (capable

of xenobiotic degradation), and can grow and survive in protists, plants, and higher animals. Although none of those characteristics are shared by members of the *M. tuberculosis* complex, much can be learned by comparative study of EM and the obligate pathogens, as the *M. tuberculosis* complex evolved from the EM.

The distinctive features of EM are listed in Table 1. These mycobacteria are normal inhabitants of natural and engineered environments including water and soil. Exposure and infection occurs when the habitats of humans or animals overlaps that of mycobacteria (e.g., showers). Both slowly (colony formation requires 7 days or more) and rapidly (colony formation in less than 7 days) growing species cause disease and share the common characteristic of being intracellular pathogens. Growth rate differences are significant, and because 16S rDNA sequences of rapidly and slowly growing mycobacteria are so distinct, it can be argued that they should be assigned to separate genera (Strahl and Urbance, 1990). As mycobacterial genomes are sequenced, action may need to be taken, particularly if they are reflected in differences in epidemiology. A list of EM species of public health significance is listed in Table 2. Although EM have been linked to disease by both epidemiologic analysis and DNA fingerprint analyses, to date there have been no measurements of microbial numbers or conditions required for infection, nor is there any information that would suggest there are differences in dose–response between species.

An extensive study was done in which 1×10^6 colony forming units (CFU) of each of 41 different isolates of *M. avium* complex (MAC), from water, soil, animals, and patients, were injected intravenously (IV) into C57Bl/6 mice (Pedrosa *et al.*, 1994). These strains demonstrated a wide range of virulence, from killing mice after 3–4 months to being completely cleared within that period. This demonstration of wide differences in virulence of *M. avium* complex strains suggests that pathogenicity may vary widely in the other EM species, almost certainly due to the broad genetic variations among strains. Host defenses against MAC are mostly similar to those against *M. tuberculosis* (i.e., critical involvement of $CD4^+$ T cells, need for interferon-gamma activated macrophages, containment of bacteria within granuloma), but have some differences, such as *M. avium*, but not *M. tuberculosis* being resistant to killing by nitrous oxide (NO) and acid (Appelberg, 2006). Defense mechanisms are thus comparable but not identical.

Table 1 Distinctive features of environmental mycobacteria

Feature	*Public health ramifications*
Hydrophobic cell envelope	Impermeable and resistant to antibiotics Slow growth and slow death Time for adaptation to novel conditions Impermeable and resistant to disinfectants Biofilm formation in tissue and on IMDs [a] Readily phagocytozed by macrophages Enriched in aerosols (transmission vehicle)
One to two ribosomal cistrons	Reduced growth rate Time for adaptation to antibiotics and anaerobiosis
Acid-resistant	Survive in stomach and phagosomal vesicles
Microaerobic	Growth at 6% and 12% oxygen

Epidemiology of Diseases Caused by Environmental Mycobacteria

Recognition of the lack of person-to-person transmission of infection by EM led to the discovery of environmental reservoirs. These reservoirs include natural and drinking waters, soils, aerosols, and dusts. Useful models for the epidemiology of environmental mycobacterial disease include *Pseudomonas aeruginosa* and *Legionella pneumophila*. Both are water- and aerosol-borne pathogens whose infections are rarely transmitted between infected individuals.

Diseases caused by EM are listed in Table 3. The classic presentation of pulmonary mycobacterial infection of older white males with lung problems due to smoking, drinking, or work in a dusty occupation has undergone two successive waves of change. Starting in 1982, the picture of mycobacterial disease was changed dramatically by the onset of HIV infection and AIDS and the ensuing surge of *M. avium* disease in AIDS patients. Following

Table 2 Environmental mycobacterial species of public health significance

Subdivision	*Environmental mycobacterial species*
Slowly growing (colony formation in greater than 7 days)	*Mycobacterium kansasii* *Mycobacterium marinum* *Mycobacterium ulcerans* *Mycobacterium avium* *Mycobacterium intracellulare* *Mycobacterium malmoense* *Mycobacterium haemophilum* *Mycobacterium xenopi* *Mycobacterium simiae*
Rapidly growing (colony in less than 7 days)	*Mycobacterium abscessus* *Mycobacterium chelonae* *Mycobacterium fortuitum*

Table 3 Diseases caused by environmental mycobacteria

Disease	*Risk factors*	*Mycobacterial species*
Pulmonary disease	COPD, pneumoconiosis, cystic fibrosis, pectus excavatum	*M. kansasii, M. avium, M. intracellulare, M. abscessus*
Cervical lymphadenitis	Young (6 months–3 years)	*M. avium*
Skin granuloma	Occupational (fish)	*M. marinum*
Skin infections	Kidney transplant	*M. haemophilum*
Buruli ulcer	Proximity to streams	*M. ulcerans*
Disseminated bacteremia	HIV-infection and immunosuppression	*M. avium*
Nosocomial	Surgical trauma	*M. chelonae, M. fortuitum, M. abscessus*

the introduction of highly active antiretroviral therapy (HAART) and some improvements in antimycobacterial therapy, and the consequent reduction of AIDS-related infections, there has been the emergence of an increasing rate of pulmonary disease in slender elderly women (*M. intracellulare, M. avium*, and *M. kansasii*) who lack any of the classic risk factors. Other current presentations of disease caused by EM include: cervical lymphadenitis in children (i.e., *M. avium*), occupation-associated pulmonary infection (e.g., Buruli ulcer, *M. ulcerans*), dermal infections (*M. marinum* and *M. haemophilum*), disseminated infection in AIDS and other immunosuppressed (*M. avium*) and nosocomial infections (*M. abscessus, M. fortuitum*, and *M. chelonae*) associated with surgical trauma.

Presentation, Diagnosis, and Treatment of Environmental Mycobacterial Infections

Pulmonary Disease

Presentation of pulmonary EM infection in immunocompetent individuals includes cough, weight loss, and night sweats (as also seen with classical tuberculosis caused by *M. tuberculosis*). There appear to be two different groups of patients, one consisting of men with predisposing conditions and the second comprised of elderly, slender women. In the latter group, exposure to potting soil or showers is associated with disease. Further, some in this latter group have mutations in the cystic fibrosis transmembrane-conductance regulator (CFTR) and α_1-antitrypsin (AAT) genes (Kim *et al.*, 2005). In light of the fact that the average age of the human population is increasing and EM occupy habitats co-occupied by humans and animals, the incidence of pulmonary disease in the elderly is expected to increase. *M. avium, M. intracellulare, M. kansasii, M. malmoense* (Europe), *M. xenopi* (outbreaks linked to hot water systems in buildings), and *M. simiae* (linked to groundwater sources) are the most common EM species detected in these individuals. Radiographic features are similar to those of *M. tuberculosis* infection (e.g., granulomatous disease). Bronchiectasis is often found in slender, elderly women and men with pulmonary mycobacterial infection. Diagnosis is based both on clinical presentation, radiographic features, and mycobacterial culture. Diagnostic criteria and guidelines for treatment have been recently revised by the American Thoracic Society and involve multidrug therapeutic regimens (Griffith *et al.*, 2007). If patients can tolerate the side effects of the drug combinations, there is a high likelihood of disappearance of symptoms and mycobacteria.

Cystic Fibrosis (CF)

Over the past 10 years it has become increasingly apparent that cystic fibrosis patients are at risk for pulmonary mycobacterial infection. *M. avium* and *M. abscessus* have been independently isolated from CF patients, and such infections do lead to deterioration of the CF patient's health status (Olivier *et al.*, 2003). A recent study of the epidemiology of *M. abscessus* infection in CF patients demonstrated that patient-to-patient transmission rarely occurred and that the likely source of infection again was the patient's environment.

Cervical Lymphadenitis

Infection of the cervical and mandibular lymph nodes, presenting as swollen glands principally in children from 6 months to 2 years, can lead to draining sinuses if untreated. Diagnosis is based on the presence of acid-fast bacteria in aspirates. Until 1985, the majority of cases were caused by *M. scrofulaceum*, but now *M. avium* is the pathogen throughout the developed world (Wolinsky, 1995). Antibiotic therapy is ineffective and surgical resection of the infected node is both effective and is not followed by reoccurrence (Wolinsky, 1995).

Disseminated Infection (Bacteremia) in AIDS and Other Immunosuppressed Individuals

HIV-infected individuals whose $CD4^+$ counts fall below 100 per ml are likely to develop opportunistic infections, such as disseminated *M. avium* infection. After *Pneumocystis*

(carinii) jiroveci (45%), *M. avium* (25–30%) is far and away the most common EM isolated from AIDS patients in the developed world. *M. tuberculosis* (41%), rather than EM, is more frequently recovered from AIDS patients in the developing world. Diagnosis is based on cultivation of mycobacteria, predominantly *M. avium* or *M. tuberculosis*, from lysed white blood cells enriched from blood samples, in conjunction with other samples (e.g., sputum, feces, bone marrow, or tissue). Guidelines for treatment of *M. avium* and EM infection have been published by the American Thoracic Society (Griffith *et al.*, 2007) and usually involve multidrug regimens. If patients can tolerate the side effects of the long (9–24 months or longer) multidrug regimen, their disease can be cured. However, if the strain becomes resistant to the primary drug, the macrolide clarithromycin, then treatment often fails. Disease typically subsides if $CD4^+$ counts can be raised above 200 by HAART or other intervention. The long-lasting macrolide azithromycin, or clarithromycin, has been recommended as prophylaxis for some individuals with HIV infection.

Dermal Infection

Skin granulomas caused by *M. marinum* are often associated with individuals exposed to fish either by occupation (i.e., commercial fishermen) or by maintaining home or office aquaria. Interestingly, the prevalence of *M. marinum* and other EM in fish is increasing, and has reached epidemic proportions (~20% of all fish caught are infected) in certain locals, such as the Chesapeake Bay near Maryland in the United States (Kane *et al.*, 2007). The cause of this is unknown, it could be possibly related to global warming. Another characteristic skin infection, the Buruli ulcer, is caused by *M. ulcerans*. Historically this disease was found principally among people living in the river courses in Uganda, but recently, the infection has appeared in Australia, again linked to proximity to watercourses. Recent evidence of detection of *M. ulcerans* in biting insects whose habitat is watercourses, coupled with knowledge that long pants and long-sleeved shirts were protective, suggests that the infection is transmitted by insect bites, the first classification of mycobacterial infection as a vector-borne disease. *M. ulcerans* has also been discovered in snails, another possible reservoir. Treatment of Buruli ulcer involves surgical excision followed by antibiotic therapy. Dermal infections caused by *M. haemophilum*, a heme-requiring mycobacterium have been reported in kidney-transplant patients. For all three species, skin localization of infection may be linked to the rather low optimal temperature for growth (28–32 °C). Dermal *M. abscessus* infections have been associated with exposure to waters (e.g., public baths) and there is growing awareness that exposure to podiatric footbaths are linked with mycobacterial (e.g., *M. abscessus*) foot infections.

Nosocomial Infection

A number of mycobacteria have been linked to nosocomial infections occurring in association with surgery or surgical trauma (Wallace *et al.*, 1998). Primarily, nosocomial infections are caused by rapidly growing mycobacteria, namely *M. abscessus, M. chelonae*, and *M. fortuitum* (Wallace, 1994). Infections have been linked by DNA fingerprinting to disinfectant solutions, ice, or water in the hospital environment or to mycobacterial-contaminated devices.

Tools for Mycobacterial Study

Because of the inherent slow growth of many EM, colonies can often be overgrown by other microorganisms in either patient or environmental (e.g., water or soil) samples. It is also likely that culture media commonly used for isolation of mycobacteria and developed for isolation of members of the *M. tuberculosis* complex are inadequate for promotion of colony formation of certain EM. Cultural and biochemical tests, analysis of cellular fatty acids by HPLC, gene amplification and restriction fragment polymorphism analysis, and 16S rDNA sequencing can all lead to the identification of EM. All have limitations, particularly with regard to an inability to distinguish distinct species whose separation may be important for treatment or DNA fingerprinting (e.g., *M. avium* vs. *M. intracellulare*). A variety of techniques and markers are available for typing EM including some of general applicability (e.g., pulsed field gel electrophoresis, PFGE) and those that are species-specific (e.g., IS*1245* and IS*1311* in *M. avium*). Although the sensitivity of detection and quantitation has not reached the limits achieved by culture isolation, numbers of EM can be measured by quantitative polymerase chain reaction (qPCR). Clinical identification of EM is best left to experienced reference labs.

General Characteristics of Environmental Mycobacteria

Genomics of Environmental Mycobacteria

A picture of the genomes of a number of EM is beginning to emerge as a result of the relatively large number of mycobacterial genome-sequencing projects that have been completed including: *M. tuberculosis*, *M. leprae*, *M. bovis*, *M. bovis* BCG, *M. ulcerans*, and *M. avium* subsp. *paratuberculosis*. Annotated sequences for *M. abscessus* and *M. chelonae*, *M. avium* subsp. *avium*, and *M. smegmatis* strain are expected. Several genomes have excellent online annotation sites (see the section titled 'Relevant Websites'). Thus, useful and valuable tools for comparative genomics and other

genetic analysis methods are available within the genus *Mycobacterium*.

The largest genotypic difference between members of the *M. tuberculosis* complex and the EM is related to horizontal gene transfer. Members of the *M. tuberculosis* complex lack plasmids and its highly stable genome has appeared to have evolved principally by chromosomal insertions and deletions. In contrast, EM genomes are far more diverse and show evidence of horizontal gene transmission. For example, *M. avium, M. intracellulare*, and *M. scrofulaceum* carry related plasmids (Meissner and Falkinham, 1986). Analysis of the genome of *M. avium* subsp. *paratuberculosis* revealed the presence of an 'island' whose base sequence was significantly different from that of the rest of the genome, suggesting transfer and integration from another organism.

Genomics has shed light on the interesting study of how members of the *M. tuberculosis* complex evolved from the EM. The genomes of the obligate, host-restricted pathogens are reduced (*M. tuberculosis* is 4.41 million base-pairs [Mbp] and *M. bovis* BCG is 4.37 Mbp) compared to the EM (*M. smegmatis* is 7.0 Mbp and *M. ulcerans* is 5.63 Mbp). While the *M. avium* chromosome is not much larger (4.7 Mbp), strains can contain up to 1.4 Mbp (30% of the total genomic DNA) as extrachromosomal plasmid DNA (Meissner and Falkinham, 1986). *M. ulcerans* also contains a 174 kb plasmid, which carries the genes for synthesis of the mycolactone toxin which is a key to pathogenesis. This plasmid has been transferred to other EM, demonstrating horizontal gene transmission, in this case of virulence traits. It appears that many of the genes related to virulence in animals were acquired by EM before the divergence of the obligate pathogens. A set of 219 genes conserved between *M. tuberculosis* and *M. leprae* and not found in other bacteria were shown to be present in EM such as the nonpathogen *M. smegmatis* and the opportunists *M. avium* and *M. marinum* (Marmiesse *et al.*, 2004). However, much is left to be discovered on the genomics and evolution of EM. Clinical and environmental surveys of EM show that, despite the naming of over 50 new species since 1990 (Tortoli *et al.*, 2003), many mycobacterial isolates still cannot be identified to the species level and belong to new, undescribed groups.

Population Genetics

The population genetics of EM vary quite widely. For example, only two of five different genotypes (clones) of *M. kansasii* are associated with disease throughout the world. In contrast, there is wide genetic heterogeneity among members of both *M. avium* and *M. intracellulare* isolated from patients. While virulence in mice (assessed by lethality and bacterial burden in organs) is generally higher in *M. avium* complex isolates from infected animals and immunocompetent patients and lower in environmental isolates and strongly immunocompromised patients (Pedrosa *et al.*, 1994), there is no specific characteristic yet linked to murine virulence, including uptake or growth rates in macrophages and dendritic cells. Thus, pathogenicity appears to be multifactorial. It is intriguing to speculate that the virulence associated with the two *M. kansasii* clones is due to accumulation of one or more specific virulence genes, whereas virulence can be associated with many different clones of members of the *M. avium* complex.

Characteristics Relevant to Ecology and Epidemiology

A variety of physiologic features of the EM are determinants of their ecology and hence epidemiology (**Table 4**). The key characteristic of environmental mycobacterial cells is their hydrophobic, lipid- and wax-rich outer membrane (Brennan and Nikaido, 1995). Accumulated evidence has shown that the long-chain fatty acids are arranged in a lipid bilayer in which are embedded porins for transport. Transport through the outer membrane is very slow and thus permeation of nutrients limits growth rates. Dedication of a substantial portion of their metabolism to the synthesis of C_{60}–C_{80} lipids also contributes to slow growth. Although slow growth might be considered a debilitating feature for a microbial cell, slow death also goes hand in hand with slow growth. Slowly growing microorganisms are more resistant to disinfectants and antibiotics, because macromolecular unbalance (e.g., cell growth in the absence of cell wall synthesis) is more difficult to achieve. Further, while EM grow slowly,

Table 4 Characteristics relevant to ecology and epidemiology

Characteristic	*Ecologic and epidemiologic ramifications*
Hydrophobic	Attachment to surfaces Concentrated at air–water interfaces Concentrated in ejected droplets Readily aerosolized Disinfectant resistance Slow growth and slow death
Oligotrophic	Growth in low nutrient (50 μg/L) environments
Microaerobic growth	Growth and survival in anaerobic environments
Slow growth	Time for adaptation to stress Slow death
Acid-tolerance	Prevalent in acidic soils and waters
Metabolize polar organics	Metabolize pollutants and organics
Humic growth stimulation	Prevalence in humic-rich environments
Temperature adaptation	Growth from 15 °C to 45 °C

their metabolism is as rapid as faster-growing microbes. Thus, they can adapt to conditions (e.g., exposure to disinfectant) and survive because of the induction of protective gene products. The hydrophobic surface also mediates (1) attachment to surfaces, (2) concentration at air–water interfaces, (3) concentration in ejected droplets, and (4) antibiotic- and disinfectant-resistance.

The mycobacterial hydrophobic surface means that they are readily aerosolized and can survive exposure to antibiotics and disinfectants. Widespread implementation of chlorination and similar disinfectants in water treatment may be a practice selecting for mycobacteria (Bland *et al.*, 2005). Further, hydrophobicity also contributes directly to their ability to metabolize a variety of polar organics, including pollutants. Example organics discovered as being used as carbon sources by EM include latex rubber, pyrene, and other polycyclic aromatic hydrocarbons; vinyl chloride; isooctane and other petroleum-derived compounds; sterols, methanol, and other alcohols; thiophenes; and benzylamine. With this evidence in hand, there is no doubt that current human practices of water disinfection and continued pollution are enlarging and enriching the habitats for mycobacteria. It is no wonder that the incidence of disease caused by EM is increasing.

EM also appear to be oligotrophic, capable of growing in low-nutrient (assimilable organic carbon [AOC] $\geq$ 50 μg/L) environments (e.g., drinking water distribution systems). Their ability to grow under microaerobic conditions and survive long term in anaerobic conditions means that this group of opportunistic pathogens can grow in still water in distribution systems and households and in tissues at low oxygen tensions. The acid-tolerance of EM explains, in part, their prevalence in acidic soils and waters and survival in the stomach. EM have been isolated from habitats of both low (Alpine) and high (coastal U.S. swamps) temperature. For example, strains of *M. avium* can grow from 15–45 °C. It is not yet known whether the alpine mycobacteria can grow at 37 °C or higher. Further, a number of mycobacterial species can survive exposure to high temperature; most notably *M. xenopi*, in which infections are usually linked to hot water systems in hospitals and apartment houses.

Ecology of Environmental Mycobacteria

EM are found in a variety of natural (**Table 5**) and engineered (**Table 6**) environments. Their presence in these habitats is dictated by those physiologic features listed previously. For example, persistence in drinking water distribution systems and household water systems (including household hot water heaters and shower heads) is due to the ability of EM to grow in waters of low organic matter content, resistance to disinfectants (e.g., chlorine and ozone), and ability to adhere to pipe surfaces and form biofilms. The preference of EM to adhere to surfaces, form, and grow in biofilms, rather than in suspension, also contributes to the ecology of mycobacteria. Mycobacterial presence in biofilms leads to increased resistance to disinfectants and antibiotics. Further, mycobacterial cells released from biofilms maintain their resistance. Growth of released biofilm cells in suspension results in the loss of that resistance. Thus, growth of mycobacterial cells in biofilms results in transient, adaptable antibiotic- and disinfectant-resistance and serves as an example of the physiologic adaptability of these opportunistic environmental pathogens that have ramifications for water treatment, waterborne EM disease transmission, and patient treatment. For example, mycobacterial cells released from pipe biofilms may continue to grow in water despite the presence of disinfectant, because they are transiently resistant. Likewise, mycobacterial cells released from catheter surfaces are transiently antibiotic-resistant. If patient antibiotic dosage was based on susceptibility of suspension-grown cells, concentrations might be too low to prevent growth of the released, transiently resistant cells, which may explain the clinical persistence despite long antibiotic treatments.

Recovery of high numbers of EM from acidic, coastal southeastern swamps in Virginia and pine forest soils, peats, and water draining from those regions in Finland, are due to acid-tolerance, growth stimulation of the humic compounds that are in high concentrations in those habitats, and the ability of the EM to grow over wide temperature ranges.

Environmental mycobacterial ecology in both natural and engineered water systems is further influenced by their interactions with protozoa and amoebae. First, environmental mycobacterial numbers correlate

Table 5 Environmental mycobacteria in natural environments

Natural environment	*Environmental mycobacteria*
Boreal, pine forests	*M. avium*
Peat bogs	*M. avium*
Potting soil	*M. avium, M. intracellulare, M. kansasii*
Brown water, acidic swamps	*M. avium, M. intracellulare*

Table 6 Environmental mycobacteria in engineered water systems

Engineered environment	*Environmental mycobacteria*
Drinking water distribution systems	*M. avium, M. intracellulare*
Showerheads	*M. avium*
Spas and hot tubs	*M. avium*
In-line water filters	*M. avium*
Ice, ice machines	*M. abscessus*
Footbaths	*M. abscessus*

with the numbers of protozoa and amoebae. Second, 26 species have recently been shown to survive in amoebae (Adelkambi *et al.*, 2006), demonstrating that this trait appears general to all EM. In other studies, the EM were pathogenic to the protozoans, or neutral or even symbiotic. EM inside vegetative amoebae or within cysts can survive antibiotic and disinfectant treatment. These infected protozoa may also serve as vehicles into animal hosts. EM directly released from protozoa are more infective in mice. Because protozoa and amoebae occupy many of the same habitats as do the EM (e.g., natural waters and water distribution systems), it is likely that these interactions influence not only mycobacterial ecology, but their ability to cause disease and resist antibiotics. This fascinating interaction deserves much further study.

A number of mycobacterial species demonstrate unique environmental distributions. For example, *M. malmoense* is principally found in Europe and *M. simiae* appears to be restricted to regions served by groundwaters from specific aquifers. The high frequency of water samples collected in the southeastern United States correlates with the fact that this same region has the highest frequency of individuals with reactivity to *M. intracellulare* protein antigens. Because disease caused by EM is not reportable in the United States, it is not known whether the environmental distribution of skin sensitivity and EM correlates with disease.

Emerging Environmental Mycobacterial Diseases

Mycobacterium ulcerans and Buruli Ulcer

Historically Buruli ulcer, caused by *M. ulcerans*, was found in a limited geographic region. Outbreaks in other regions of the world, including Australia, led to increased interest in the epidemiology of this disease. Although a complete description of its epidemiology is still lacking, a number of important factors have been identified. For example, it has been shown that the mycobacterium can be detected in environmental samples including soil, water, and insects. Earlier work had established that risk factors for Buruli ulcer included exposure to watercourses and that long pants and sleeves were protective. A current plausible hypothesis is that Buruli ulcer is a consequence of an insect in a watercourse, carrying *M. ulcerans*, biting an individual in that habitat.

Mycobacterium immunogenum and Pulmonary Disease in Metal Workers

Outbreaks of hypersensitivity pneumonitis (HP) in metal workers in the automobile industry have been linked to the presence of EM in the metal-working fluid. A current working hypothesis is that the mycobacteria (e.g., *Mycobacterium immunogenum*) are introduced during the dilution of the neat metal-working fluid in water and that the use of disinfectants to retard microbial growth and degradation of the properties of the metal-working fluid leads to the selection of the disinfectant-resistant mycobacteria. EM have been shown to be capable of the use of a number of components of metal-working fluid (e.g., tall and pine oils) for growth. HP in the workers is not primarily infection, but rather immunological overreaction to mycobacterial cell wall products.

Diseases of Possible Mycobacteriology Etiology

Two diseases of unknown etiology, sarcoid and Crohn's disease, have been proposed to be due to EM infection or indirect effects thereof. In part, this hypothesis has been spurred by the fact that EM cause granulomas and these two are granulomatous diseases. Little evidence is available to either support or refute the hypothesis that sarcoid is of mycobacterial etiology, despite long-standing interest and many studies.

Although evidence to judge whether Crohn's disease is caused by a mycobacterium is being published, the data are insufficient to come to any definitive conclusion. Interest has focused on *M. avium* subsp. *paratuberculosis* in Crohn's disease, because that mycobacterium causes Johne's disease in cattle. Johne's, like Crohn's, is a disease of the GI tract and causes chronic diarrhea and wasting in cattle and ruminants. Recovery of *M. avium* subsp. *paratuberculosis* as colony-forming cells from either Crohn's disease tissue or environmental samples is infrequent and incubation before colony formation is prolonged. *M. avium* subsp. *paratuberculosis* has been detected in patient tissue, milk, and environmental samples by PCR amplification of its unique insertion sequence, IS*900*. Isolation from milk reflects the resistance of EM to elevated temperature. Although the frequency of detection in Crohn's tissue is higher than that from normal individuals, the relatively high frequency of isolation from normal controls prevents clear proof of the hypothesis. Antimycobacterial therapy trials, although successful in some patients, have been unsuccessful in others, again preventing clear proof. It has been proposed that the organism's presence in water reflects its transmission from feces of infected dairy herds and waterborne transmission. Further, the ability of *M. avium* subsp. *paratuberculosis* to survive in protozoa and amoebae suggests a mechanism of transmission.

Future Picture of Environmental Mycobacterial Disease

As described above, the epidemiology of environmental mycobacterial disease has changed and continues to change. Currently, with *M. avium* disease in AIDS

patients under control because of HAART and effective antimycobacterial therapy, the traditional picture of disease in only men with predisposing conditions is being augmented by the increasing incidence of pulmonary disease in slender elderly women (and men). In addition, cervical lymphadenitis in children continues with the change that *M. avium* has been recovered since 1985 and *M. scrofulaceum* disappeared as the causative agent (Wolinsky, 1995). In addition, a large number of new species have been described, whose epidemiology and ecology are completely unknown. The description of the species *M. immunogenum* followed reports of its recovery from metal-working fluid and linkage with automotive workers with hypersensitivity pneumonitis. These workers are exposed to aerosols of metal-working fluid and a number of the outbreaks have followed the disinfection of the metal-working fluid, leading to killing of other microorganisms and likely resulted in the proliferation of mycobacteria in the competitor-free environment.

It is likely that the incidence of environmental mycobacterial disease will continue to rise, promoted in part by an increasing demand for new water sources whose quality may not be as high and the selection for EM in engineered water delivery systems by disinfection. Thus, increased numbers of EM will be present in environments occupied by humans. As the average age of the human population in developed nations increases, it is likely that the incidence of mycobacterial pulmonary disease will rise. The incidence of disseminated mycobacterial disease may also rise because an increasing proportion of individuals will be transiently or permanently immunosuppressed because of cancer, chemotherapy, or immunosuppression coincident with transplantation. It is also possible that the presence of mycobacteria in foods (e.g., *M. avium* subsp. *paratuberculosis* in milk and meat) may lead to additional disease in humans.

Public Health Measures to Prevent Environmental Mycobacterial Disease

A number of measures may reduce the possible exposure of humans and animals to EM. First, because the ultraviolet (UV) susceptibility of the EM is similar to other waterborne microorganisms, replacing chlorination with UV irradiation of water may reduce mycobacterial numbers. Second, household and other point-of-use water filtration can reduce numbers of mycobacteria. Care must be taken with this recommendation because it has been shown that mycobacteria can grow in the filters. Ultimately if the filter is not changed, it can become a source of mycobacteria. Proof of identical DNA fingerprints of shower water and patient isolates suggest that closed showers and long shower aerosol exposure may be a risk factor for pulmonary mycobacterial disease. Reduction of dust exposure when working with peat-rich potting soils is also a possible risk reduction measure (by wetting the soil).

See also: Adenoviruses; Arboviruses; Bacterial Infections: Overview.

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Environmental Protection Laws

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Introduction

The manufacturing, processing, and use of chemicals, materials, tools, machinery, and equipment in industrial, construction, mining, and agricultural workplaces often cause environmental, health, and safety hazards and risks. Occupational and environmental factors cause or exacerbate major diseases of the respiratory, cardiovascular, reproductive, and nervous systems and cause systemic poisoning and some cancers and birth defects. Occupational and environmental disease and injury place heavy economic and social burdens on workers, employers, community residents, and taxpayers.

Because voluntary efforts in the unregulated market have not succeeded in reducing the incidence of these diseases and injuries, the public has demanded government intervention into the activities of the private sector. This intervention takes the form of the regulation of environmental health and safety hazards through standard setting, enforcement, and transfer of information authorized by legislation. This article addresses the major regulatory systems (or regimes) designed to protect public and worker health from chemicals discharged from sources that pollute the air, water, ground, and/or workplace. The establishment of standards and other legal requirements in these regulatory regimes has occurred over a more than 30-year period that has seen changes in the use of scientific and technical information in regulatory initiatives and in legal doctrine, including the manner in which science, economics, and technological capability are viewed by the courts. The concepts of risk assessment, cost–benefit analysis, and technology forcing have evolved, both through the development of case law and through changes in the political environment. Often, changes in one of the regulatory regimes has affected the other regulatory regimes as well.

Standards can be classified in a number of ways. A performance standard is one that specifies a particular outcome – such as a specified emission level above which it is illegal to emit a specified air pollutant – but does not specify how that outcome is to be achieved. A design or specification standard, on the other hand, specifies a particular technology – such as a catalytic converter – that must be used. In either case, the standard can be based on (1) a desired level of protection for human health or environmental quality, (2) some level of presumed technological feasibility, (3) some level of presumed economic feasibility, or (4) some balancing of social costs and social benefits. Within each of these options, there is a wide spectrum of possible approaches. A human health-based standard, for